AIDS
TO
MICROSCOPIC DIAGNOSIS
(BACTERIAL AND PARASITIC DISEASES)

E. BLAKE KNOX

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BAILLIÈRE, TINDALL & COX

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BY

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PREFACE

THE object of this handbook is to supply those preparing for examinations with a work for revision purposes, of a convenient size to read in an easy-chair or to carry about. It is not intended to serve as a substitute for the many excellent text-books on Bacteriology, Parasitology, or Tropical Diseases which should always form the student's first line of defence against what may be termed the 'active chemiotaxis' of the examiner.

Modern medical examinations are now so intimately bound up with the pros and cons of microscopic diagnosis that the student must give to this subject his closest consideration, for knowledge of it will provide him with a trusty weapon that will not fail in time of need. The questions 'How would you investigate the cause of this disease?' or 'How can you confirm your clinical diagnosis?' may in a very large proportion of cases be answered—'By the microscope.' In practice, the causal agent of a disease having been diagnosed, the line of further action is clear.

While every attempt has been made to bring the text up to date, it is hoped that the errors of commission will not outweigh those of omission, as the writer's space is limited, and the subjects discussed have necessarily to be confined to an epitomized account of the more important matters bearing on examination questions and laboratory work.

No attempt is made to claim originality in the subjects treated ; the book is a compilation of notes, taken in the laboratories of distinguished teachers, for whose original ideas and methods an acknowledgment of appreciation must be recorded : these are Sir Almroth Wright, M.D., F.R.S., of St. Mary's Hospital, London ; Colonel Sir William B. Leishman and Major W. S. Harrison, R.A.M.C., of the Royal Army Medical Corps College, Millbank ; and Professors A. C. O'Sullivan and A. White, of Trinity College and the Royal College of Surgeons, Dublin, respectively.

Finally, the writer's best thanks are due to S. Orson, Esq., and to his brother officers, Captain B. S. Bartlett and Lieutenant R. C. Priest, M.B., R.A.M.C., for their kind assistance in reading over the proof-sheets.

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CHAPTER I

THE BLOOD — PATHOGENIC BACTERIA AND OTHER PARASITES—THE PARASITES OF MALARIAL FEVERS

BLOOD may be examined either (*a*) fresh, (*b*) fixed and unstained, or (*c*) stained. Blood examined fresh should always be ringed with vaseline to keep it from drying, and if it be diluted with an equal quantity of $\frac{3}{4}$ per cent. salt solution, to which a little methyl-violet has been added, the nuclei of leucocytes, blood-plates, nucleated red corpuscles (normoblasts), and parasites, are brought out. The lashing movements of spirochætæ (relapsing fever, etc.) can be well demonstrated by this method, as also can the sluggish movements of filaria sanguinis hominis. Where recognition of the changes in the size and shape of the red blood-corpuscles is desired, blood may be examined, fixed but unstained, a 2 per cent. solution of osmic acid being placed on the patient's finger, and the skin pricked through the drop. The fixed blood is now examined on a slide. The examination of blood in film is best made on slides, as they have the advantage of being easily manipulated, and a greater area of blood is available. Probably the simplest method of spreading is to use a spreader (a slide with one corner cut off). The drop of blood is placed at one end of the slide, and the spreader is brought up to the drop at

an angle of 45 degrees, so that the drop of blood comes to occupy and fill the angle. The spreader is now brought, quickly but steadily, away from the drop along the slide, avoiding pressure or lifting, and the blood forms a thin, even, ribbon-like layer on the slide.

The advantage of having the drop behind the spreader, and not in front of it, is that the blood-cells, etc., are not crushed by the opposing glass surfaces. When the blood-smear is stained and examined, it will be found that the majority of the leucocytes lie at the free edge of the blood-ribbon. Films may be fixed by corrosive sublimate if staining by methylene blue and eosin or other ordinary staining methods are desired. But as Leishman's stain (see Chapter VIII., p. 132) has the advantage of fixing and also staining, it is by far the best, quickest, and most efficient method of staining; it is a permanent stain which differentiates so sharply and clearly that it is unnecessary to use an oil-immersion lens for most clinical work, unless bacteria are to be looked for, and the films can be examined with a $\frac{1}{6}$ or $\frac{1}{8}$ objective.

THE PARASITES OF THE BLOOD.

Amongst the parasites which may be found in the blood, representatives of both the vegetable and animal kingdom occur.

I. Vegetable Parasites.

This group represents the fission fungi (schizomycetes), otherwise known as bacteria and cocci, the more important, occurring to a less or greater degree, being the bacilli of anthrax, tubercle, glanders, typhoid fever, plague; the bacillus pyocyaneus, B. coli communis; and cocci—micrococcus melitensis, streptococci, and staphylococci. The question of the occurrence of the bacillus of tetanus is doubtful.

Methods of Examination.

(*a*) *By Direct Examination.*—Blood-smears are made on slides, and deeply stained by basic aniline dyes. As a

rule, the bacteria occur in such very small numbers that this method will fail to demonstrate the organisms in a single drop of blood. If, however, a few cubic centimetres of blood are drawn from the patient, and an equal volume of a solution of citrate of soda in distilled water be added to prevent clotting, and this be centrifuged, smears of the sediment, suitably stained, will often demonstrate the organisms.

(*b*) *By Culture.*—Draw off a few cubic centimetres of the blood under aseptic conditions and inoculate into broth flasks or tubes, incubate for twenty-four hours, centrifuge the sediment, and stain smear preparations. This is a very reliable method.

Bacillus of Anthrax.—Found in anthrax and woolsorter's disease. Splenic blood gives the best results in man, in whom the bacilli always appear in less numbers than in animals. If bacilli are in small numbers, diagnosis may possibly be obtained by injecting the suspected blood into a guinea-pig or mouse. If the disease is present, the bacilli will develop in large numbers in the animal's blood. In blood from a living animal the bacilli do not occur in long threads, nor do they form spores. (For diagnostic details, see Chapter III., p. 65.)

Bacillus of Tubercle.—This organism may be extremely hard to demonstrate in the blood-stream, as when present it occurs in very few numbers. Forsyth has recently recorded a method by which he demonstrated tubercle bacilli free in the peripheral blood in cases of non-acute pulmonary tuberculosis. He drew 5 c.c. of blood from any convenient vein of the forearm, and at once mixed it with an equal volume of sterile citrated salt solution. The mixture was centrifuged for twenty minutes, and a thick smear made of some of the sediment on a slide. This was dried in an oven at 60° C., and was then placed in distilled water to lake the blood. The film was next fixed and stained in the usual way. Rosenberger claims to be able to demonstrate the bacilli in the

blood of every patient, whatever the type of the disease, and however early the disease clinically. (For general diagnostic details, see Chapter IV., p. 80.)

Bacillus of Glanders.—Found in the farcy buds and ulcers of glands, and also at times in the blood. Löffler's staining method (*vide* Chapter VIII., p. 132) is advised. (For diagnostic details, see Chapter III., p. 60.)

Bacillus of Typhoid Fever.—The presence of the bacillus typhosus in the blood is a most important confirmation of early diagnosis, as one can obtain a definite diagnosis before it is possible to get Widal's reaction, or perhaps to obtain the classical signs and symptoms of the disease. With proper technique the bacilli can be isolated in every case, the chief factor being to take a *sufficient* quantity of blood (1 to 3 c.c.). Blood may be drawn from any vein; the median basilic is usually selected. The blood is then placed in a *large* quantity of culture media (100 to 200 c.c. broth), and incubated at blood-temperature, and the sediment plated. Recently, using better media (ox bile), incubating at blood-temperature, and plating on Fawcus's medium (*vide Royal Army Medical Corps Journal*, February, 1909), the method has been much simplified, and the results have been exceedingly good. Daily blood-cultures can also be used for aiding prognosis, as in ordinary uncomplicated cases the bacteria get fewer and fewer in the blood from day to day as the disease progresses. In what is likely to become a severe case they persist in large numbers, and are still found in the fourth week in the blood. (For diagnostic details, see Chapter V., p. 83.)

Bacillus Coli Communis.—In cases of peritonitis and infection from wounds of the intestines recent research has shown that this organism occurs in the blood. The sediment of centrifuged blood inoculated into bile-salt broth (McConkey's), and then plated on bile-salt-lactose neutral-red-agar and incubated at 37° C. for twenty-four hours, should show red colonies on the surface. These

can be bacteriologically confirmed by inoculation into the 'flaginac' media. (For details, see Chapter VII., p. 113.)

Plague Bacilli. — These are present in the blood in some cases before the buboes appear ; in other cases they only appear in the blood during the late stages of the disease. A thick blood-smear is stained after removing the hæmoglobin with distilled water, or 5 to 10 c.c. of blood is placed in broth and incubated for twenty-four hours. Sediment falls to bottom, leaving the broth clear. The sediment is examined. Puncture of early buboes gives best results.

Bacillus Pyocyaneus.—This may occur as a causal agent in forms of endocarditis, especially in children. (See Chapter III., p. 52.)

Micrococcus Melitensis.—This occurs in the blood early in the disease in cases of Malta fever. Some freshly-drawn blood is taken (2 to 3 c.c., or the cocci may be missed) and inoculated into nutrient broth ; incubate, and examine. Confirm diagnosis by agglutination reaction. (See also Chapter V., p. 83.)

Other Micro-organisms occurring in the Blood.—Beside those already enumerated, many others occur. In inflammatory endocarditis, streptococci, pneumococci, the influenza bacillus, gonococcus, staphylococcus albus, in order of frequency, have been found, as well as in their own special diseases. The best method to demonstrate these is to inoculate 2 to 3 c.c. of blood in broth ; incubate, and examine.

II. Animal Parasites.

Under this heading are included the parasites causing malarial fevers of the protozoal class, as well as others, such as leishmania, trypanosomata, spirochætæ, distomata, filaria, etc.

THE PARASITES OF MALARIAL FEVERS.

Malarial fevers are protozoal diseases (protozoa being the lowest members of the animal kingdom), the particular

protozoa concerned belonging to the genus *Hæmosporidia*, the parasite having been discovered by Laveran in 1880. The parasites have been found in man (three varieties), monkeys (one variety), birds (two varieties, Halteridium being one), bats and frogs (one).

Varieties found in Man :

1. Hæmamœba malariæ (parasite of Quartan Fever).
2. Hæmamœba vivax (Tertian Fever).
3. Hæmamœba præcox (Malignant or Æstivo-Autumnal Fever).

Morphological Characters Common to all Malarial Parasites :

1. Affect the red blood-corpuscles of host, living in them at their expense, and finally destroy them.

2. Colourless masses of protoplasm ; no cell wall.

3. Motile at certain stages of their life. Two forms of movement—(*a*) amœboid by means of pseudopodia ; (*b*) circulatory or streaming, seen in the pigment granules.

4. Form a pigment (melanin granules) at some time of their existence from the hæmoglobin of the red cells they affect.

5. Have a definite period of development, varying from twenty-four to seventy-two hours.

6. Have two methods of reproduction : (*a*) Asexual, by segmentation or simple division ; rosette in body of man, birds, etc. (warm-blooded host). (*b*) Sexual, like fertilization of ovum by spermatozoa, which commence in body of warm-blooded host, and are completed in body of mosquito or other carrier (mosquito gametes).

7. Possess a definite staining reaction :
   - (*a*) Protoplasm blue by methylene blue or Romanowsky's stain.
   - (*b*) *Chromatin* granules bright ruby red with Romanowsky's stain.

8. All possess *chromatin*, the amount and distribution of which is a means of telling the varieties of parasite from each other and one stage from another. Chromatin and protoplasm are found in all protozoa if alive.

The Asexual Cycle or Cycle in Man.—Three main changes:

1. Entrance of spore into red corpuscle.

2. Growth at expense of red corpuscle; protoplasm increases; melanin granules form; finally, segmentation occurs by formation of sporocytes or rosettes.

3. Rupture of red corpuscle; spores escape and fix on to other red cells; melanin taken up by large mononuclear leucocytes.

THE ASEXUAL CYCLE DESCRIBED.—1. The spores are first free in the blood-plasma. They are small round masses of protoplasm, having one or two chromatin dots with a central clear space, and are known as the *young ring form* (euchœmospore). This attaches itself to the edge of a red corpuscle, flattens itself out, and protrudes thin arms, or pseudopodia, which dig into the corpuscle. At this stage it has a 'humped-back' appearance. Finally, the entire parasite gains entrance through the pseudopodia. Probably the sexes can be made out at this stage in malignant tertian fever.

2. The ring (euchœmospore) now grows at the expense of the red cell, its protoplasm increases, melanin granules form, and pseudopodia are thrown out. Two paths of further development are now open: (*a*) sexual cycle; (*b*) asexual cycle.

3. More melanin and protoplasm are formed; a half or three-quarters of the red cell's diameter is occupied; it finally fills almost the entire red corpuscle, and segmentation occurs as a rosette.

4. In forming a rosette the chromatin splits up into 2-4-6-8 masses, according to the variety; each mass surrounds itself with protoplasm; the melanin collects in clumps at the centre or sides, and each segment is known as a *sporocyte*. The rosette bursts, rupturing the red corpuscle, and the sporocytes pass free into the plasma, of which nothing is now left but its skin and particles of melanin. The large mononuclear leucocytes take up the melanin, and carry it to the spleen, liver, or skin.

5. The sporocytes, which have some slight motility, now fix on to fresh red cells, and the cycle begins over again.

The asexual cycle takes twenty-four, forty eight, or seventy-two hours to complete, and the setting free of the sporocytes indicates the height of the fever.

The Sexual Cycle.—This commences in the body of the warm-blooded host (man), and is completed in the body of the mosquito or other carrier. The changes described in the early stages of the asexual cycle (see 1 and 2) hold good, but the clear zone in the centre of the young ring form (euchœmospore) persists later, and there is no cleavage or division of either protoplasm or chromatin. The growth of the latter goes on increasing until the entire red cell (except a thin rim) is filled up. The fully-formed sexual forms are completely speckled with melanin. At this stage they are differentiated into a male gamete (microgametocyte), which exflagellates, acting as a spermatzooon, and a female gamete (macrogamete), acting as an ovum. For fertilization to occur it is necessary that both sexes be taken into the stomach of a mosquito or other carrier, as it cannot take place in a warm-blooded host.

SEXUAL STAGE IN THE MOSQUITO.—Fertilization having occurred in the stomach of a mosquito, the parasite becomes an elongated actively motile *travelling vermicule*, swims away to the stomach wall, and, penetrating the epithelial cells, gets to the outer wall between the longitudinal and transverse muscle fibres; here it comes to rest, and is known as an *immature zygote* (7·5 μ long).

The Immature Zygote.—This has a firm capsule, inside of which changes occur similar to those taking place in the yolk of an egg. The protoplasm divides into a number of little spores, the pigment lying in one corner of the capsule; the whole body increases rapidly in size (50 μ), and is now known as a *zygotomere.*

The cellular contents now develop into 5 to 7 or 8 to 10

balls (*blastophores*, or *blastomeres*), these being spherical masses with hairy coats (like little balls of wool) inside the capsule of the zygotomere. The walls of the blastophore now break down, and the capsule is full of thread-like appendages, and is known as a *mature zygote*. It takes about ten days to arrive at maturity.

The *Mature Zygote* ruptures and sets free its contents into the body cavity of the mosquito, and these are known as *sporozoites* or blasts.

The *Sporozoites* pass (probably by way of the blood) to all parts of the mosquito's body, being motile, and get into the salivary glands and ducts. When the mosquito bites man or other warm-blooded host, it injects these into the blood, and here they form rings (stages up to ring not known), and act as the spores do in the asexual rosette, and form a new cycle.

CLASSIFICATION OF MALARIAL FEVERS.

I. Benign Tertian.
II. Quartan.
III. Malignant Tertian.

I. Benign Tertian Malaria.

1. This is the commonest variety of malaria, the red cells affected soon becoming swollen, enlarged, pale, and often distorted.

2. The protoplasm of the red cells degenerates, forming *Schüffner's dots*. These are granules which stain a brilliant red with Leishman's stain ; they are found outside the parasite in the red blood-corpuscles, having no connection with the body of the parasite, and when present are diagnostic of benign tertian, being only found in it.

3. Amœboid movements of the parasite are rapid, and circular movements also occur.

4. The parasite fills the whole cell when fully grown, forming fine yellow-brown pigment.

5. Rosettes are not often found in the peripheral blood, but occur in the internal organs. They contain eighteen to twenty-four spores in the fully-formed rosette, filling the entire red corpuscles.

6. The sexual forms are circular. Gametes are not found early in first infections; they take about a week to develop. This is important, as patients *without* gametes are easily cured by quinine, and are not infectious.

7. The *female gamete* has its chromatin as a dark-staining mass, surrounded by a vacuole; this, again, is surrounded by pale blue protoplasm, containing *fine* pigment, the whole arrangement being neater and more elegant than occurs in the male gamete.

8. The *male gamete* has its chromatin paler and more diffuse, protoplasm 'dirtier-looking,' and its pigment coarse and brown.

9. *Relapses* are due to gametes in the blood. Formation of a rosette as an offshoot of a parasite in a red cell is called *gameto-schizont* or *gamete-splitter* (Shoupin).

10. Duration of cycle from the time the rosette bursts (which is *supposed* to coincide with the rigor of the patient), to the time of the next rigor is forty-eight hours. If rigors or fever occur daily instead of every second day, it is due to other crops of spores maturing. The spores breaking up and discharging their toxin causes the rigor. During the 'hot' and 'sweating' stages the *young* parasites are seen in the red cells, and leucocytes may be seen carrying pigment. It is not the presence of the parasite that causes the fever, but the presence of the *sporulating* parasite.

11. *Gametes* vary in number; males are rarer than females. Owing to propagation by mosquito, it is very important to recognize the gametes, and so prevent the spread of the disease. They are found in the blood between the attacks. It is not certain if quinine has any action on them, but it has on the rosettes, young ring forms, and especially on free spores.

II. Quartan Malaria.

Said to be the malarial fever of cold climates, but it is found in some of the hottest places—*e.g.*, West Coast of Africa, where it is distributed in little patches over large areas. Its chief characteristics are :

1. The miniature form of the parasite with a large amount of pigment, and the red corpuscles not being enlarged or swollen, not losing colour, and having *no degenerative* changes, such as Schüffner's dots.

2. The rosette has only eight to twelve spores, arranged in a circle round a mass of pigment in the centre.

3. The pigment is very coarse, very dark, and very abundant.

4. Amœboid movements slow.

5. The period of development is seventy-two hours, but the typical quartan chart is rare, a double or treble quartan infection being generally found.

6. The patient has a large, hard, chronic spleen.

7. The sexual differences of the gametes are similar to the benign tertian parasite, but they do not swell up the cell, and have more pigment.

8. They do not take the colour away from the red cells.

9. Being the mildest type of malaria, it yields readily to quinine.

III. Malignant Tertian Malaria.

Other names : Pernicious Malaria, Remittent Malaria, Tropical Malaria, Subtertian Malaria (Manson), Crescent Malaria, Æstivo-Autumnal Fever.

THE PARASITE is characteristic.

1. Instead of being spherical, it forms small rings, and the gametes appear as crescents. Both are found in the peripheral blood in all stages of the attack, and persist for a long time—two to three weeks.

2. In the peripheral blood it appears as a ring or a streak across the red cell, and only fills up about half the cell.

3. Never grows to any size.

4. Only in very exceptional cases are rosettes found in peripheral blood.

5. Red cells not swollen, rather tending to shrink or become crenated.

6. Red cells do not lose their colour ; the hæmoglobin tending to concentrate, makes the corpuscles darker in colour—a bronzed appearance : hence the name 'brassy bodies.'

7. Occasional presence of *Maurer's dots* in sexual forms. These are supposed to be scar areas of the red cells, wounded by the retracting movements of the arms of the parasite ; they appear as bright red streaks with Leishman's stain, and can only be demonstrated by heavy staining. They increase in numbers as the parasite grows older.

8. *Pigment* very abundant ; coarser and darker than benign tertian, but similar to quartan. As a rule, none is found in the peripheral blood, except in crescents.

9. In the deep vessels *rosettes* are found, very neat little bodies, having a centre of black pigment, surrounded by very small spores. These *spores* are sometimes as small as the Malta fever coccus. In some forms of malignant tertian twelve spores occur in the rosettes ; in others up to thirty-six spores are found.

10. Differentiation of the rings which proceed to gamete formation :

(*a*) No bulging of the chromatin mass, and its diameter is the same as that of the protoplasm.

(*b*) Dark-red staining capsule round the parasite, a sort of membrane.

11. *Gametes.*—Actual development not known, and is an unsettled point. *Male* shows dirty protoplasm. The *female* occurs as a neat-cut crescent ; protoplasm sky-blue ; chromatin has a ring of black pigment around it. The male is a large, clumsy, kidney or oval sausage-shaped parasite ; protoplasm dirty mauve colour ; chromatin

pale-staining and diffuse, may fill up the crescent. *Pigment* coarse and brown. In some cases the red cell has been outgrown by the parasite. If the nuclear chromatin bulges, it is probably an asexual form.

Sexual Differences of Gametes as Demonstrated by Leishman's Stain.

	Female.	Male.
Form ...	More crescent-like	Reniform, bean-shape, or oval
Protoplasm	Clearer and more hyaline, sky-blue colour	Granular, dirty mauve colour
Chromatin mass	Small dense clump in centre, bright staining	Large, faintly staining, may fill up crescent
Pigment ...	Lies in wreath around the chromatin	Irregular mass around and over the chromatin

12. Exflagellation of the male crescent can be studied on a moist slide; it only occurs during life. The crescent loses its form, and becomes oval and then spherical. The pigment is distributed in a ring; it shoots out arms four or five times longer than the gamete, actively motile, which break off and swim away. This occurs only in the body of the mosquito, or outside the human host.

13. Malignant tertian fever is the most chronic and longest infective type of all forms of malaria. It does not react to quinine like quartan. Relapses believed to be due to gameto-schizonts. Pernicious complications of different types occur, such as coma. This has symptoms like heat-stroke, and the parasites are found swarming in the brain capillaries; the red cells lose their elasticity, and block the capillaries. In the Peshawar type the same conditions prevail, as well as a choleraic form, in which the patient has vomiting of blood or bloody diarrhœa, due to capil-

laries becoming blocked with parasites. In black-water fever similar complications occur, but hæmoglobin instead of blood is passed in the urine.

Other Blood-Changes in Malaria.

1. There is no initial leucocytosis.

2. Between the attacks there is an increase in the large mononuclear cells up to 15 to 25 per cent. This is also seen in trypanosomiasis and in kala-azar.

3. Pigmentation of leucocytes (mostly large mononuclears). A mass of melanin granules in one of these is positive evidence of malaria. Not often seen in the peripheral blood, and is generally carried and deposited in the internal organs (spleen and liver).

DIFFERENCES OF QUARTAN, BENIGN, AND MALIGNANT TERTIAN FEVERS TABULATED.

	Quartan.	Benign Tertian.	Malignant Tertian.
Asexual cycle	72 hours	48 hours	About 36 hours
Amœbulæ movements	Slow	Active	Very active
Pigment and movements	Coarse, non-motile	Fine; Brownian movements	More intense, dark and coarse, non-motile
Sporocyte ...	$\frac{2}{3}$ size of red cell	Larger than red cell	$\frac{1}{3}$ size of red cell
Red cells ...	Normal size	Increased in size, swollen	Shrunken
Colour ...	Normal	Lost or *pale*	Not pale; brassy
Rosette ...	Daisy, 9 spores in peripheral blood	Big, sunflower shape; lot of spores (18): spores in peripheral blood	Small rings, perhaps crescents; irregular daisy shape; 8 to 12 spores; very rare in peripheral blood, but in organs
Gametes ...	Oval	Oval	Crescents, not spheres; female, sky-blue, male, dirty protoplasm
Schüffner's dots ...	Absent	Present	Absent
Maurer's dots	Absent	Absent	Present
Infection ...	Single or double	Single or double	Double commonest

CHAPTER II

LEISHMANIA—TRYPANOSOMATA—SPIROCHÆTÆ—DISTOMATA—FILARIA

Leishmania.—This name has been provisionally given as a generic nomenclature to a sub-order of flagellated protozoa having a parasitic existence in man. Nothing is as yet definitely known either of their precise position in the protozoal kingdom or concerning their natural development outside the human body, beyond the suspicion that they may possibly be transmitted by blood-sucking insects from some intermediary host, which in the case of kala azar is thought to be the Indian bed-bug (*Cimex rotundatus*). Under the heading Leishmania, three supposedly different diseases occur, which are caused by three *supposedly* different parasites respectively.

1. Kala-azar, or 'black sickness,' occurring chiefly in India, and caused by *Leishmania donovani.*
2. Oriental sore, or Delhi boil, occurring chiefly in India, and caused by *Leishmania tropica.*
3. Infantile splenic anæmia, occurring chiefly in Algiers, and caused by *Leishmania infantum.*

L. donovani (Kala-azar).

For many years kala azar in India baffled all attempts to elucidate its causal agent. It used to be regarded as a modified form of malarial cachexia, until Colonel Sir William Leishman, R.A.M.C., in 1900, discovered the protozoal parasite with which his name and that of Donovan have been associated.

Microscopic Diagnosis.—As the parasites are not found, as a rule, in the peripheral blood, but occur in great

abundance in the spleen, liver, and bone-marrow, for diagnostic purposes resort has to be made to splenic puncture—an operation not without grave risk.* In carrying this out, the skin and instruments having been scrupulously sterilized, the patient is warned to avoid movement. A dry syringe and small needle must be used, as any extraneous moisture will cause the parasites to become spoiled. Splenic cells, avoiding blood as much as possible, should be withdrawn quickly, and a pad and firm binder applied to the wound; the patient should lie quite still in bed for several hours afterwards. Film preparations on staining with Leishman's stain will show parasites in abundance; they appear lying typically intracellular as minute round, oval, or pyriform bodies, about the size of a blood-plate, measuring from 2 to 3 μ long, and 0·5 to 1 μ broad. Their protoplasm, which is hard to demonstrate by staining, may show vacuoles, and contains two chromatin nuclei, one being large, compact, and staining faintly, the other small and rod-like and staining deeply. The parasites are typically intracellular, lying inside the endothelial cells of the bloodvessels and in leucocytes; very occasionally are they found free. The disease is associated with chronic fever, cachexia, enlarged spleen, discoloration of the skin; hence its name, 'kala-azar.' Marked anæmia is only characteristic of the later stages. Blood-examination shows extreme leucopænia, which is very marked and almost pathognomonic, the leucocytes being often down to 3,000, or even below 1,000 (relative leucopænia is less marked during high fever), increase in mononuclears and lymphocytes, and decrease in polynuclears and eosinophiles.

CULTIVATION.—From spleen-pulp mixed with citrated blood at room temperature the parasites can be made to grow larger; they develop vacuoles, and splitting occurs at the micronuclear end. A pale mass appears, from

* To increase coagulation of the blood, the internal administration of 15 grains of calcium chloride t.i.d. for a few days, before puncture, is recommended.

the latter a flagellum grows out, and the flagellated body now appears like one of the developmental stages of a trypanosome.

Distribution of Kala-azar.—Headquarters Assam, spreading along the east coast of India and Madras ; also recorded from China, Penang, Philippines, Sudan, Algiers, Mediterranean, and Crete.

Spread of Infection.—This is supposed to be by the common blood-sucking Indian bed-bug (*Cimex rotundatus*), or possibly transmitted by human fæces, as ulcers of the intestines have been found to contain the parasite. The life-cycle of the parasite is said to possibly consist of three stages—pre-flagellate, flagellate, and post-flagellate. The flagellate stage occurs in the mid-gut of the Indian bed-bug, the post-flagellate in the rectum of the adult host, where encystment occurs, and these cysts are ingested by the larvæ or nymphs of the insect. Indian bed-bugs become infected by feeding on the blood of kala-azar patients. There is no doubt that the parasite of kala-azar (*L. donovani*) and its two allies (L. tropica and L. infantum), as seen in man, are but stages in the life-histories of a flagellate organism living elsewhere. Captain Patton, I.M.S., has recently inoculated dogs in Madras with L. donovani in the same way as Nicholle (see p. 19), but was unable to recover the parasites. In Madras apparently dogs do not harbour them.

Prophylaxis.—The investigations connected with kala-azar do not seem to have received in this country the recognition which they merit, but it is a matter for congratulation that through them it has been already possible to apply preventive measures in infected localities in India, by burning down bug-infested huts and removal of the infected villagers. Experimental treatment by atoxyl in large doses is recommended, and, from the fact that the Tunisian parasite has been found in dogs, the possibility of a curative serum derived from this source is suggested.

L. tropica (Oriental Sore, or Delhi Boil).

This is a specific, benign, chronic, intractable ulceration of the skin of the hands, forearm, or face of a granulomatous nature, due to a tropical protozoal parasite closely allied and very similar to that of kala-azar, and which is inoculable. Smith, of the Army Medical Service, first described Delhi boil in the A.M.D. Report, 1868. Colonel Firth, R.A.M.C., in 1891 described the parasites, and also noted that he found similar bodies in the ulcer of a dog's back in India. Since then numerous forms of similar ulcers have been described from different parts of the world, such as Aleppo boil, Veldt sores (due to staphylococci), Scinde sores, etc., the pathology of which requires further investigation.

MICROSCOPIC DIAGNOSIS.—The parasites are similar to the Leishman-Donovan bodies found in kala-azar—small round or oval bodies 2 to 3 μ in diameter. They are best found in scrapings from the chronic granulations of the ulcers; they stain well by Leishman's stain. As many as twenty round or oval sharply-defined bodies lying in one cell have been found. These on treatment by Leishman's stain show a peripheral pale blue portion and a central part which stains badly. There are also two chromatin masses—one large, occupying almost a quarter of the cell lying near its periphery, and a small rod-shaped micro-nucleus, which stains deeper. A flagellated stage of development, somewhat similar to that of *L. donovani*, has been described by Row.

GEOGRAPHICAL DISTRIBUTION.—Endemic localized areas in India, Arabia, Persia, Asia Minor, North Africa, Crete, Crimea, South America, etc.; said to be more common in towns than in the country, and more prevalent at the beginning of the cold weather.

SOURCE OF INFECTION.—Not known. Probably by flies or other biting insects. It is a disease of camel-using countries, and affects dogs and other animals. In

man it is contagious and auto-inoculable; incubation period three to twelve days. One attack confers further immunity, and it is thought virulence to man may be removed by transit through animals. If this be so, it is suggestive of the possibility of a vaccine.

PROPHYLAXIS.—The Jews of Bagdad prevent disfiguration by inoculating their children on the buttocks.

Leishmania infantum (Algerian Infantile Splenic Anæmia).

This is a recently-described obscure form of splenic anæmia occurring in young children in Algiers, clinically resembling the cachexia of kala-azar, and associated with enlarged spleens. The parasite, which differs very little from that of kala-azar, has been found by Nicholle and Comptè, and it appears to grow on MacNeal's medium very easily. There is a history of sick dogs being associated with the children, the latter afterwards developing the disease, which is suggestive of a canine origin, more especially since Nicholle and Comptè have found considerable numbers of the same parasite in the spleen, etc., of a Tunisian poodle which suffered from purulent otitis, and they produced further infection in dogs by inoculation. Nicholle thinks transmission from dogs to man probably occurs by means of fleas.

THE TRYPANOSOMATA.

The trypanosomata are a group of protozoal flagellated organisms found as parasites in the blood of vertebrates, and causing a form of disease known as trypanosomiasis. The following varieties have been recognized:

1. *Tr. lewisi* (found in rats).

2. *Tr. evansi* (the cause of surra in cattle, horses, and camels in India).

3. *Tr. rougeti* or *equiperdum* (the cause of dourine amongst horses in the Mediterranean littoral).

4. *Tr. brucei* (the cause of tsetse-fly disease, or nagana,

in herbivora, especially horses, cattle, etc., in South Africa, and pathogenic to all animals, except large game, which are tolerant).

5. *Tr. theileri* (found in African cattle).

6. *Tr. ugandense* and *Tr. gambiense*. *Tr. hominis* (the cause of sleeping sickness of West and Central Africa, and now believed to be the same organism).

7. *Tr. sanguinis* (frog).

MICROSCOPIC DIAGNOSIS, ETC., OF THE TRYPANOSOMATA.—If blood containing trypanosomes be examined, they will be found to present the following characteristics:

1. The parasite is always free in the liquor sanguinis and never intracorpuscular. It is a minute, fusiform, somewhat fish-like unicellular mass of transparent, colourless protoplasm, which shows minute granulations in its body, and two chromatin masses, termed respectively, the micronucleus, lying at the posterior end, and the macronucleus, at the anterior end. A lateral expansion or flattening is termed the undulating membrane. The body tapers towards the ends, and has a pointed flagellum, starting at the micronucleus, and this, turning back, comes to lie along the body of the organism, and becomes free at the anterior end, from which end the parasite moves forward by lashing its flagellum and by an undulating movement of its protoplasm. There may be a vacuole near the micronucleus.

2. SIZE.—Varies from about 14 to 30 μ long and from 1·5 to 3 μ broad.

3. MULTIPLICATION occurs by fission. The micronucleus first splits, then the macronucleus, and then the flagellum; finally, the entire parasite splits from the anterior end. Sexual forms have been described, those with darker-staining protoplasm being considered females. Minchin has described a form of encystment as occurring in the stomach of biting flies, the parasite before encystment, so to speak, swallowing its flagellum, and rolling up into a spherical encapsuled mass.

4. ARTIFICIAL CULTURE.—Some forms of the trypanosomata have been cultivated outside the body, these being Tr. lewisi, Tr. evansi, and Tr. brucei. Mackie and MacNeal's media give good results, but the parasites grow quite unlike what they appear in the blood, and resemble elongated kala-azar organisms, or spherical and rosetted bodies; they lose their virulence very rapidly in culture.

5. VITALITY.—Trypanosomes, if taken away from the living body, die, being especially susceptible to warmth. They live longer when kept on ice.

6. DISTRIBUTION.—Occurs in mammals, fish, birds, and reptiles, different vertebrates having types of their own. Tr. lewisi commonest, being found in 30 per cent. of sewer rats without causing them disease. Tr. brucei causes tsetse-fly disease, or nagana, in cattle and horses in South Africa. Wild game may be infected without getting sick. Tr. evansi causes surra in horses, cattle, and camels in India; it is supposed to be inoculated from the bite of a fly, and the parasite is very similar to Tr. brucei. Surra gives rise to a form of remittent fever, with local patches of œdema of the skin, prepuce, etc.

7. IMMUNIZATION. — If an animal be immunized against Tr. brucei, it can be inoculated with Tr. evansi and get the latter disease, and *vice versa*—*i.e.*, it can only be made immune to one class of disease by inoculation with the particular parasite of that disease.

Sleeping Sickness.—This disease was worked at on the Congo, and its parasite described by Dutton in 1901, and Todd later, the parasite being named Tr. gambiense. Castellani in 1902 found it in centrifuged cerebro-spinal fluid in a case of sleeping sickness in Uganda, but failed to appreciate its significance until Sir David Bruce, R.A.M.C., in 1903, clearly demonstrated its presence in every case of sleeping sickness, and inoculated *rhesus*

monkeys with the parasite. Bruce, working on the theory of his discovery of the fly agency of cattle disease in South Africa, further found that the distribution of sleeping sickness in Uganda exactly corresponded with the distribution of a blood-sucking fly, the *Glossina palpalis*, a species closely allied to the *G. morsitans*, or tsetse fly, which is the spreading agent of the nagana cattle disease in South Africa. Sir David Bruce, R.A.M.C., got reports from each district in Uganda, and had flies collected, and prepared spot maps as to the distribution of glossinæ and as to the prevalence of sleeping sickness. These maps proved that the distribution of the glossinæ and sleeping sickness were almost identical, and corresponded to a small belt composed of marshy creeks. Experimentally it was shown that monkeys bitten by glossinæ fed on infected material developed sleeping sickness, and trypanosomes were found in their cerebrospinal fluid. Monkeys were also infected by glossinæ caught wild.

PROPAGATION.—This was for a time difficult to decide in countries where there are numerous blood-sucking insects full of various forms of parasites. It was thought until quite recently that infection was spread by purely mechanical inoculation by *G. palpalis*, the fly being a greedy feeder and sucking up a large quantity of blood. Colonel Sir David Bruce, R.A.M.C., has recently, however, definitely confirmed Kleine's experiments, and we may now regard *G. palpalis* as the host of *Trypanosoma gambiense*, in which the latter undergoes a cycle of development, and consider that the infective process is not a mere mechanical transmission. The developmental forms, like the rosettes, etc., which occur in artificial culture, are also found in the proboscis of the fly.

G. palpalis, it seems, relies chiefly on human blood for its sustenance, though upon occasion, when this is not available, it is content to feed on the blood of

other vertebrates, including the crocodile, as Koch has pointed out. It has a preference for biting the black rather than the white man, and it shows a similar preference for settling on dark garments rather than on white. The flies follow in the wake of native caravans, resting, when necessary, on the persons of the native carriers or on their burdens. They accompany men on board boats or vessels on rivers and lakes, and in this way are transported long distances. They have been known to enter railway carriages, and to travel by this means hundreds of miles from their original haunts, hidden underneath the seats, and obtaining their meals by biting the bare legs of native passengers. For this reason it is feared that the construction of new lines of railway which pass through infected districts may give rise to serious danger by assisting to diffuse the disease. The natural enemies of *G. palpalis* are not yet known, but it is believed that some fungi attack them, and that insectivorous animals, including bats, birds, ant-eaters, and reptiles, feed upon them. It has been discovered that the fly has a marked repugnance to certain plants—*e.g.*, citronella. This grass repays for its cultivation by the essential oil which it contains, and areas planted with it are avoided by the flies. Since sleeping sickness depends for its spread on three factors—the fly, the trypanosome, and the human being—preventive measures should include attacks upon the fly in its haunts; removal of healthy persons from its vicinity, or their protection from its bites; the prevention of access of the trypanosome carriers—*i.e.*, infected human beings—to fly areas; and, lastly, the treatment of such persons by medical remedies which free the peripheral blood and perhaps permanently rid it of parasites.

Distribution in Man.—In man the trypanosomes are found in the blood, and also in small numbers in the cerebro-spinal fluid, which it is necessary to centrifuge in order to demonstrate them. They are found early and

late in the disease in the glands, more especially the cervical glands, which become enlarged. Puncture and extract juice, and they can be found. This is the method of choice for diagnosis of early cases, and isolation of carriers will prevent spread of the disease.

PATHOLOGY.—*Post-mortem* special lesions are to be ound in the brain.

1. Infiltration of leucocytes occurs all round the small vessels.

2. Endothelial thickening of bloodvessels, lumen constricted, and they contain little or no blood.

3. Chronic meningo-encephalitis.

4. Dura mater very adherent to calvarium and brain.

5. Cerebro-spinal fluid increased and turbid at times.

The only other condition in which these lesions occur is in general paralysis of the insane, and they are indistinguishable in the two diseases. No trypanosomes have, however, been found in these lesions. Perhaps they get into the cerebro-spinal meninges, but not into the brain itself.

Glossina palpalis.—This is a small fly, varying in size, generally about the size of an ordinary house-fly, but differing from the latter in having a very markedly developed biting apparatus in front of the head, and by sitting with its wings folded, not separated. It lays one pupa at a time, under stones or cover in dark places, liking the shade of water-plants, as sunlight dries and kills the pupa. There is a very great danger of sleeping sickness spreading wherever the *G. palpalis* occurs. Up to the present few flies are infected, but as the flies are distributed over large areas, the danger is of utmost importance.

SYMPTOMS IN MAN.—Patient gets what is apparently an attack of malaria, which does not yield to quinine. Irregular attacks of fever follow, and a marked cachexia develops with—

1. General œdema, especially of the face and lower eyelids.

2. General congestion of the skin, with cyanotic infection in areas and patches of erythema, which disappear and reappear.

3. General wasting, muscular weakness, splenic enlargement, and increased pulse and respiratory rate.

The symptoms are usually very insidious in onset (rarely start as an epileptic attack or mania). Patient becomes listless and morose; full and puffy face; drooping of upper eyelids and protrusion of lower lip; gets headache, attacks of giddiness, and gastro-intestinal disturbances. Later he gets very exhausted and disinclined for work, and drops asleep at odd times; likes to lie sleeping in the sun. This lethargy grows gradually worse; he secludes himself, and becomes silent or taciturn. If made to walk, he does so as if half awake or drunk. Temperature generally subnormal. With diminished muscular power he gets muscular tremors. Later on he falls asleep while eating; he loses flesh; muscular tremor of hands and tongue well marked; may get convulsions, followed by paralysis, bedsores, swollen lips, saliva dribbling away. The other signs generally seen are enlargement of cervical glands, itchy papules, vesicular eruption (especially on chest).

Duration of the disease is from four months to four years, and it attacks both sexes and all ages from three years upwards.

Diagnosis from Beri-Beri.—Sleeping sickness is a disease of the central nervous system; beri-beri affects the peripheral nerves.

First Stage.—Trypanosomiasis (enlarged glands, fever, anæmia, and œdema of face).

Second Stage.—Sleeping sickness.

Eighty to ninety per cent. of the natives of Uganda get trypanosomiasis, but only a few develop sleeping sickness.

SPIROCHÆTÆ.

This is an important group of organisms, which were formerly classed as bacteria, but are now considered as belonging to the protozoal kingdom. The following is a more or less complete list of the best known spirochætæ, following Lühe's classification after Leishman.

(A.) True Spirochætæ.

1. *Spirochæta plicatilis.*—The type species. A water organism, which may reach a length of 200 μ, and in which Schaudinn has demonstrated the presence of an undulating membrane and the absence of flagella.

2. *S. balbianii.* — Found in the intestines of the oyster.

3. *S. dentium.*—A minute organism found in the mouth and dental tartar of man.

4. *S. buccalis.*—A large organism found in the same sites as *S. dentium.*

5. *S. Vincenti.*—Found by Vincent in a form of pseudo-diphtheritic sore throat, and also in hospital gangrene.

6. *S. vaccinæ.*—Found in vaccine lymph.

7. *S. refringens.*—In syphilitic ulceration and other lesions of skin and mucous membranes.

8. *S. pseudo-pallida.* — Often found in association with *S. pallida.*

9. *Spirochæta of dysentery.*—Described by Le Dantec.

(B.) Blood Spirochætæ.

1. *S. anserina.*—Found in geese by Sakharoff.

2. *S. gallinarum.*—Found in fowls in Brazil.

3. *S. obermeieri,* vel *recurrentis.* — The cause of relapsing fever.

4. *S. duttoni.*—The causal agent of African relapsing, or tick fever.

5. *S. carteri.*—A very small spirochæta, causing Asiatic relapsing fever.

6. *S. novyi.*—The causal agent of American relapsing fever, spread by ticks.

7. *S. theileri.*—Found in Transvaal cattle.

8. Additional spirochætæ have been found in the horse, sheep, bat, Norway rat, bandicoot, cockroach, mosquito, and tsetse fly, which may possibly belong to this group.

(C.) TREPONEMATA.

1. *Treponema pallidum*, vel *Spironema pallidum*, vel *Spirochæta pallida.*—The causal agent of syphilis, discovered by Schaudinn and Hoffmann in 1905.

2. *T. pertennuis.*—The causal agent of yaws, discovered by Castellani.

Relapsing, or Spirillar Fever (Spirochæta obermeieri).

This is a specific contagious disease, associated with overcrowding, destitution, or famine, in warm or temperate climates, usually epidemic, with fever without any rash, lasting for about a week, and followed by a relapse after an interval of about another week. The disease is due to a spirochæta, discovered in the blood by Obermeyer in 1873, and called *Spirochæta obermeieri.*

MICROSCOPIC DIAGNOSIS.—This is made from the blood, which should be taken during pyrexia before the crisis, as the organisms are absent during the apyrexial intervals, but appear again during recurrent attacks. The *Spirochæta obermeieri*, vel *S. recurrentis*, until 1904 was considered to be a bacterial spirillum, but Schaudinn showed it to be protozoal. The parasite is a long thin thread, measuring from 16 to 40 μ in length, by 1 μ in width, pointed at the ends, spirally coiled, with bold flowing curves, which show a certain amount of regularity. It is motile, often producing vigorous corkscrew-twisting movements round a long axis ; these movements may

reverse themselves. There is also a movement of progression, the organism at times appearing to dash across the field under the microscope. Bending movements also occur. Some observers think this motility is due to flagella (lateral or terminal), but latest views (Schaudinn) point to the absence of flagella, and suggest that the motion is due to the organism possessing an undulating membrane, like that possessed by the trypanosomes, and which is extremely difficult to demonstrate by staining. *Staining* is effected by watery solutions of the basic aniline dyes; better results are obtained by Leishman's stain. They are Gram-negative. Attempts at *cultivation* in artificial media have resulted in failure. The spirochæta has been successfully *inoculated* in man, monkeys, white mice, and rats. Novy and Knapp have kept spirochætæ alive through many generations in white rats and mice by intraperitoneal inoculation. During the disease in man, germicidal, immunizing, and agglutinating substances are formed in the blood, from which it is probable that serum-therapy will be applicable.

CHANNEL OF INFECTION, ETC.—This for a long time was supposed to have been by the medium of bugs biting; now the body louse is thought to be the transmitting agency. After seven days' incubation relapsing fever reaches its climax on the sixth or seventh day; then crisis occurs, followed by a fever-free period. The first attack lasts three to twelve days (mean duration $6\frac{3}{4}$ days), then an interval of two to eleven days. Second attacks occur in 77 per cent. of cases; mean duration 4·3 days, then an interval. Third attacks occur in 63 per cent. of cases. Each attack has a sudden rise of temperature, which falls by crisis. Tongue usually clean. Jaundice due to hæmolysis very common—comes on third to fifth day. Vomiting of green and black matter. Slight delirium. Spleen enlarged and palpable. A severe bilious type sometimes met with, not usually, however, in the first attack, but in the second. Mortality of the

severe bilious type 70 per cent., and of the ordinary type from nil to 12 per cent. Serum diagnosis will give some idea as to the probability of relapse. Treatment is on general lines. Quinine has most effect.

African Relapsing Fever, or Tick Fever (Spirochæta duttoni).

This is a widespread disease in Central Africa, ranging from the Congo to German East Africa. The spirochæta was first found in Uganda by Greig and Nabarro in 1903. Dutton and Todd worked at it later on the Congo, the former dying while carrying out his investigations.

MICROSCOPIC DIAGNOSIS, ETC.—This is made from the blood in the same way as relapsing fever. The spirochæta is indistinguishable from *S. obermeieri*, but is said to be slightly longer, the spirals to be wider, and the flagella to be more diffuse. The disease is milder (mortality 30 per cent.), shorter, and its attacks less frequent than ordinary relapsing fever. *S. duttoni* is spread by the bite of the African tick (*Ornithodorus moubata*) to man, and from tick to tick as far as the third generation. According to recent work by Colonel Sir William Leishman, R.A.M.C., the infection probably spreads indefinitely. The disease can be reproduced by inoculating infected blood into monkeys, rats, etc. Immunity conferred by the spirochæta of tick fever does not confer immunity to diseases produced by other varieties of spirochæta.

Syphilis (Treponema pallidum, vel Spirochæta pallida, vel Spironema pallidum).

This protozoa, foreshadowed by Donné in 1837, was definitely isolated and described by Schaudinn and Hoffmann in 1905, and is now regarded as the specific organism of syphilis. An important feature linking it to

protozoa is the great similarity of the histological lesions of the nervous tissues of trypanosome infections (sleeping sickness and dourine) to syphilitic lesions. Dourine (*mal de coït*) may almost be regarded as equine syphilis, as its lesions and clinical symptoms are very similar to human syphilis. Again, mercury and arsenic are specific for trypanosomata and spirochætæ infections.

MICROSCOPIC DIAGNOSIS, ETC.—The organism is very minute and difficult to find, even when present in large numbers. It is spiral-shaped, showing from six to eight curves, which are small, sharp, rigid and regular. Long forms occasionally occur. It measures from 4 to 14 μ in length, and is extremely thin, 0·25 μ. The spirals are very regular and closely twisted throughout, except at the ends, which are tapered to a point. There may or may not be a delicate flagellum at each pole. It is doubtful if an undulating membrane is present; Schaudinn thinks not. From the top of one curve to the top of the next the measurement is 1·1 μ, and the depth of each curve is 1 μ. Thus seven curves represent the diameter of a red blood-corpuscle, and a good method of differentiating this spirochæta from others occurring in syphilis (*S. refringens* and *S. pseudo-pallida*) is to bear in mind the approximate distances between the curves. In fresh preparations it exhibits three kinds of movement—rotation on its long axis, flexion, and gliding or progressive motion.

DETECTION IN FRESH PREPARATIONS.—The best method is to transfer scrapings of chancres, condylomata, buboes, or squeezed-up lymph after puncture, to a 0·7 per cent. sodium chloride solution; prepare a hanging drop from the centrifugalized sediment of this, and examine microscopically with a Leitz dark ground condenser and a Nernst lamp or other brilliant light.

STAINING.—Difficult. Requires special methods, which are described on pp. 134 to 137.

CULTURES.—Have been unsuccessful, except those by collodion sac-culture method in animals.

Inoculation Experiments in Animals.—Metchnikoff and Roux were the first to demonstrate experimentally the communicability of syphilis to animals, and to show that the nearer the animal approached to man the closer the disease approached in its characteristics and virulence to the human form of the malady. Thus, though other animals, especially anthropoid apes, can be successfully inoculated, the chimpanzee alone reproduces with absolute certainty the human symptomatology (primary sore and secondary lesions), the blood precipitating reaction, and recovery of the spirochætæ. Bertarelli has been able to inoculate the spirochætæ into the cornea of rabbits, and has transmitted them through a series of these animals. Other animals—guinea-pigs, cats, and sheep—have proved susceptible if inoculated in the cornea, or into the anterior chamber of the eye, and the spirochætæ have been found in the infected tissues afterwards. Levaditi has infected the eyelid of an anthropoid ape from the cornea of an infected rabbit.

It may be noted that Neisser was unable to inoculate animals by injecting the virus of syphilis into the blood or organs, but scarifying an epiblastic skin surface and rubbing in the virus proved successful, and experiments with the *Trypanosoma equiperdum* gave almost precisely similar results. As regards therapeutics, mercury and arsenic are specific for both trypanosomata and spirochætæ affections, though not of much use in bacterial infections. Mercury, especially in the form of inunction, is specially valuable, and this may be due to its preventing the pullulation of the spirochætæ on the surface of the body, a habitat which these organisms find particularly favourable for perpetuation of their species by transmission to another individual.

Although no vaccine has yet been successful for syphilis, it will no doubt come in time.

Distribution. — Schaudinn and Hoffmann have proved that the *Spirochæta pallida* is found in all the

primary and secondary lesions of syphilis, occurring in greatest numbers in the more infective lesions—condylomata, mucous patches, etc.—but never in any other disease. Being few in numbers, requiring skill in staining to demonstrate—and endurance, often for hours, in hunting sections—they are difficult to find. They occur in the deeper parts of chancres and papules, and also in the tissues, as a rule confining themselves to the depths of the lesion in the spreading inflammatory zone rather than near the surface. In the juice of enlarged or shotty glands, when the surface is unbroken, deep massage and squeezing are necessary before withdrawing lymph. After puncture allow the flow of blood to subside, and then squeeze up the lymph. The spirochæta is also found in the walls or neighbourhood of small bloodvessels and lymphatics; it is not intracellular. It is only occasionally found in the blood (septicæmia spirochæta), its conditions being apparently unfavourable for development, possibly because the organism is an anaerobe. It also occurs in great numbers in the internal organs (lungs, liver, and spleen) of infants affected with congenital syphilis, and in the placenta and umbilical cord.

Propagation in the Body.—At the seat of inoculation the spirochætæ undergo rapid multiplication, and definite histological changes take place before there is any indication of a primary syphiloma. After local development they soon reach the nearest lymph glands, where they again multiply and cause adenitis. From these they pass through the thoracic duct, and a general infection of the blood-stream occurs. Lang and Mott have noted early in the disease, before healing of the primary sore, symptoms pointing to meningitis; and Mott has observed many severe and intractable cases of brain and spinal syphilis within twelve months of infection. Hoffmann has shown that the cerebro-spinal fluid may be infective. Tertiary syphilis being as a rule non-infective, the spirochætæ are usually absent, or present in small numbers in a few cases;

possibly they exist in a latent, attenuated, or intracellular form, until raised into activity by some exciting factor (cold, trauma, toxæmia, etc.). The spirochætæ have only been found in a few instances in tertiary syphilis, chiefly in gummata of the liver. Hoffmann inoculated an ape from a gumma which occurred in a man three and a half years after the primary sore. Another interesting point is that in severe syphilis the spirochætæ may occur in very few numbers, showing apparently that the numerica factor bears but little relationship to the severity of the attack. The possibility of a resting stage, in which the ova or embryonic forms of spirochætæ may occur, should not be lost sight of. Possibly the modern microscope is not powerful enough to demonstrate their presence, or that they require special illumination. The failure to find even the adult spirochætæ by experienced bacteriologists has been used as an argument against their being the causal agent of syphilis, but their minute size, and being difficult to see and stain, should readily explain such failure.

Spirochæta Refringens.

In nearly all syphilitic lesions, especially in ulcerative sores, other spirochætæ may be found, the commonest being *S. refringens* (refractile), which, although resembling *S. pallida*, is larger, thicker, less rigid, and has longer and more irregular curves, two of which equal four of *S. pallida*. A good method of differentiation is to measure the distance between the curves. The organism stains easier than *S. pallida* by all methods; it colours blue-black by Leishman's stain. It does not cause syphilis.

Spirochæta Pseudo-Pallida.

This is also found in syphilis, and occupies a position between *S. pallida* and *S. refringens* in size, the curves being less close and regular than in *S. pallida*, and thicker and staining more deeply. Some observers think it may be a dead or a degenerated form of the *S. pallida*.

Yaws (*Treponema* vel *Spirochæta Pertennuis*).

For a long time there was thought to be some relationship or even identity between yaws and syphilis. The former is, however, an epidemic disease, and the latter pandemic. The organisms are practically indistinguishable. The *Treponema pertennuis* was discovered by Castellani in 1905 in the secretion of ulcers from yaws. It is difficult to stain. Best results are obtained by Leishman's method, allowing the undiluted stain to act on the film for five to ten minutes, and the subsequent mixture with distilled water to act for half an hour. Monkeys can be inoculated from men, and the infection becomes a general one. That yaws and syphilis are different diseases in man is proved by the fact that patients suffering from yaws can be inoculated with syphilis. Robertson thinks flies (*Musca domestica*) can transmit yaws.

Vincent's Angina, or Specific Sore Throat (*S. vincenti*).

This spirochæta is frequently found in specific sore throat (Vincent's angina), which is somewhat similar in appearance to a diphtheria throat, and is very infective. It is associated with pyorrhœa alveolaris. The spirochæta is longer and larger than *S. pallida*, and occurs in great numbers. A fusiform bacillus is closely associated with it, and the spirochæta is probably developed from it.

DISTOMATA.

The distomata, or flukes, belong to the *Trematode* order of worms (vermes) and the class *Platoda.* The diseases they produce may be included under the general term distomiasis. Two chief varieties of the parasites have been observed in human blood. These are :

1. *Schistosomum hæmatobium*, discovered by Bilharz in 1852—syn. *Bilharzia hæmatobia,* vel *Distomum hæmatobium*—causing endemic hæmaturia, termed bilharziosis,

the habitat of the adult being in the portal venous system.

2. *Schistosomum japonicum*, discovered by Katsurada in 1904, but first brought prominently to notice by Catto, and sometimes called after him as *Schistosomum cattoi*, the habitat of the adult being the smaller arteries of the intestine (G. E Brooke).

BILHARZIOSIS (*Bilharzia hæmatobia*, vel *Schistosomum hæmatobium*).

A flat worm, or trematode, causing a chronic endemic disease, giving rise to cystitis, hæmaturia, proctitis, etc., and characterized by the presence of ova in either the urine or fæces, or in both .

DISTRIBUTION.—Egypt, Sudan, Uganda, Tunis, Gold Coast, Natal, Arabia, Syria, Persia, Cyprus, Mauritius, China, America. Cases have also been recorded in the United Kingdom in people who have never been abroad.

ÆTIOLOGY.—The parasite—a trematode, or fluke worm—is an exception to the general rule of trematodes, in that the sexes are distinct. The adult resembles a round worm, owing to the fact that its lateral flattened edges are incurved or folded to form the gynæcophoric canal, in which the female partially rests after maturity. The head has two sucking discs, one being the mouth, or oral leading to the gastro-intestinal tract, and the other a ventral disc, used for fixation purposes. The *male* parasite is white, short, 1 cm. long by 1 mm. in breadth, thicker than the female, and its body is studded with tubercles. The *female* is rather darker, longer, 2 cm., twice as long as the male ; has a dark angular zigzag stripe (due to intestine being full of blood) running down its body. Two oviducts from the ovary end in a common duct at the ventral sucker. The ova, about sixty, are found in a single row, with their spines pointed back-

wards in the utero-vaginal canal. The middle portion of the body is generally infolded in the gynæcophoric canal of the male, and its head and tail lie free. The genital openings of both sexes are halfway up the body, just posterior to the ventral sucker. The sexes live apart until maturity. The worms inhabit the portal system, also mesenteric and splenic veins, and plexus of veins in the vicinity of the bladder, uterus, and rectum; they have also been found in the vena cava. In these places the female lays eggs, which block the small vessels, bursting into the tissues, and causing great irritation by their terminal spikes. The young worms are chiefly found in the *portal vein*.

TYPES OF DISEASE PRODUCED. — Two types of disease are found, and it is questionable if they are not produced by two different varieties of parasites:

1. Bladder affection (South Africa, etc.). In this the ova have terminal spikes.

2. Rectal affection (Egypt). In this the ova have a spike, situated laterally, and the symptoms produced are intestinal. There is also another variety, *Schistosomum japonicum*, found in Japan and China.

In the form affecting the *bladder* the muscular wall becomes thickened; papillary excrescences occur; ulceration sets in, with deposit of urates on the surface. The ova pass out into the urine, and give rise to hæmaturia, bright red blood being passed at the end of micturition. Very severe chronic cystitis occurs, which may spread upwards causing thickening of the ureters, pelvis of kidney, and formation of pus or calculi in the kidney. In the latter the centre of the calculus is formed of bilharzia. Masses of ova may also occasionally be found in other parts of the body, liver, lungs, etc., due to females losing their way in the blood-stream and ovulating. In the *intestinal* form, chronic fibrosis and papillary growths occur in the intestine, and the symptoms are those of a dysenteric type.

The *ova* are large, 120 to 190 μ long, by 50 to 73 μ broad, a pointed spine at one end, inside segmentation of yolk. Sometimes the embryo can be seen. When hatched out, the embryos move rapidly. When passed in the urine, they are not capable of further development. If hatched out in warm water, the embryo is seen to be covered with ciliary threads. These are especially noticeable around the mouth, and a short gastro-intestinal system, with a pear-shaped gland on each side, is visible. At the posterior end somatic, or germinal, cells may be seen, which go on to further development.

Beyond this stage, up to the development of the mature parasite, nothing is known. Theories of propagation from analogy with other trematodes suggest that the intermediate host may be a crustacean (snail), but there is no evidence to prove it. Infection in man may possibly be similar to what occurs in the case of *Ankylostoma duodenale* by means of the skin.

Infection is known to be associated with water, probably by bathing. The Zulus and Kaffirs in South Africa protect their penis with a shell, and when bathing tie a string around it.

The ordinary (urinary form) causes no danger to life. The Egyptian form is, however, dangerous.

TREATMENT.—No treatment appears to do any good. Sometimes the disease tends to cure itself spontaneously. Ova have been passed fourteen to fifteen years after infection and after leaving the infected area. Infected men are not fit for the strain of active service, but the Egyptian authorities do not invalid for it.

BLOOD CHANGES.—1. No very great anæmia; probably no toxic elements are present.

2. Eosinophilia exists up to 50 to 60 per cent.

3. Total number of leucocytes unaltered to any great extent.

SYMPTOMS.—These are due to the irritation caused by the ova. They are chiefly connected with the bladder

the sharp spikes causing irritation, laceration of tissues and hæmorrhage (diapedesis, with possibly stoppage of the blood to the part and sloughing).

PROGNOSIS.—A chronic cystitis which cannot be removed or cured. The life of the parasite depends on the life of its host.

FILARIÆ.

FILARIASIS.—This name has been given to the disease caused by the presence of filariæ, which live parasitically, chiefly in the serous cavities and the subcutaneous connective tissues. The worms are very long slender nematodes, the males being smaller than the females. The male has a bent tail, with small wing-like fins, protruding beyond which are two spicules; these emerge near the four pre-anal papillæ. In the female the vulva lies near the anterior extremity of the nematode.

PATHOGENIC FILARIÆ.—*F. medinensis*, *F. bancrofti*, *F. nocturna*.

NON-PATHOGENIC FILARIÆ. — *F. loa* vel *diurna*, *F. perstans*, *F. demarquaii*, *F. ozzardi*, *F. magalhaësi*, *F. philippinensis*.

FILARIA MEDINENSIS. — The guinea-worm occurs chiefly in West Africa, where nearly all the negroes are affected; it is also found in Tropical Africa and the Sudan, along the coastline of the Caspian and Red Seas, Persian Gulf, Turkestan, India, and possibly Fiji.

The larvæ are filiform flattened bodies, tapering posteriorly, and measure about 5 cm. long. Parturition extends over fourteen days, during which it is inadvisable to extract the worm from the tissues, as the uterus, if ruptured in the body, causes sloughing by infection of the subdermal layers with larvæ. A good mode of killing the parasite is to inject 1 in 1,000 bichloride of mercury with a hypodermic syringe either into the body or coils of the worm, or as close as possible into what is felt to be the worm. It has been suggested

that the worm by some means instinctively gets to know when water is in the vicinity, and times its parturition, selection of site of boil (legs for natives and buttocks for Europeans, as they use baths), and time of 'happy event' accordingly !

FILARIA BANCROFTI.—This nematode worm inhabits the thoracic duct and the larger lymphatics of the trunk and extremities. The sexes are distinct. The *male* worm measures about 40 mm. long and 0·1 mm. in diameter. The *females* measure 76 to 80 mm. long and 0·21 to 0·28 mm. broad. The mouth is triangular, having two lips, behind which lie six papillæ. The body is cylindrical, and the tail has a small terminal hook, which is ventrally turned up. The *larvæ*, which are motile, pass by the lymph to the blood-stream. Six or seven adults are usually found coiled up together in the lymphatics of the trunk or limbs. The adult filariæ are long thread-like worms, white in colour and smooth, tapering at the ends, and are actively motile. The *female* is twice as long and as thick as the male ; it is viviparous (giving birth to viable embryos, *Filaria nocturna*, and not ova). The geographical distribution of *Filaria bancrofti* is widespread, in Samoa and parts of Southern India nearly 50 per cent. of the inhabitants being infected.

PATHOLOGICAL LESIONS.—Two main types of disease occur : (*a*) varicosity of lymphatics by a single worm or a bunch of worms ; (*b*) abscess formation. The results produced may be stated as—

1. Chyluria (milky urine) and chylocele, due to rupture of a lymphatic varix.

2. Blocking of lymphatics, causing lymph varix, lymph scrotum, orchitis, and chylous dropsy of tunica vaginalis of testicle.

3. Inflammation of lymphatics over a large area, causing permanent thickening, and producing elephantiasis arabum, etc., associated with fever. This is a some-

what obscure disease, as some observers think it is not produced by filariasis.

4. Filarial abscesses.

FILARIA NOCTURNA.—This is the name given to the embryos of *F. bancrofti* which are found in the *blood*, and must not be confused with the adult nematodes. The parasite is about as broad as a red blood-corpuscle (so that it can get through the small blood-capillaries), and forty times as long. The head shows four to five little projections, and from the extremity of the head is a little fang. The mouth is provided with spines or spicules, which can be rapidly protruded and withdrawn. Near the head and tail are triangular-shaped spots, the anterior representing the alimentary system and the posterior the genitals. Some time after birth the embryo develops an outer sheath or cover, which appears too large for it and projects at the ends. Owing to this limiting sheath a young worm cannot perform true progressive movements, and motility depends on the flow of the blood or lymph stream, in which they show a peculiar periodicity, in that about nightfall they migrate to the peripheral vessels, and return to the deep vessels of the body and lungs the following morning. No embryos are found in the peripheral blood during the daytime, unless the host keeps awake at night and sleeps during the day, when the periodicity is inverted. No explanation can be given to account for this nocturnal migration, but it is suggested that it occurs to facilitate the taking up of the embryonic worms from the body of their host by nocturnal blood-sucking flies, chiefly the mosquito, certain species of which—*Culex fatigans* (commonest), *C. ciliaris*, *C. pipiens*, and anopheles, *P. costalis*, *Myzomyia rossii*, *Myzorhynchus siniensis*—have been proved to act as intermediate hosts. The embryos cannot reach maturity in man without passing into an intermediate host; the mosquito phase being necessary for further existence. When taken into the mosquito, their movements become rapid;

they burst their enclosing sheath, penetrate the mosquito's stomach wall, reach its thoracic muscles, come to rest, develop and increase in size, and after about ten days make their way to the proboscis of the mosquito, where they lie in pairs, and when the mosquito bites man they infect him. The heat of human blood appears to be the factor determining their exit from the proboscis at the moment of biting. If an infected mosquito be made to bite a banana, the filariæ will not leave the proboscis, and if the same mosquito be made to bite a man, the embryos will pass out. From the time of entry into man to the adult stage as *F. bancrofti*, the stages of development of the filaria are unknown.

MICROSCOPIC DIAGNOSIS.—In fresh blood taken after midnight (ringed with vaseline to keep it from drying) the embryo worms can be seen with a low power moving about amongst the red corpuscles. For permanent preparations spread the blood fairly thick and use Leishman's stain. The chief points to note are: (1) the presence or absence of a sheath and the shape of the extremities; (2) the nature of the movements and the time when found in the peripheral blood.

FILARIA LOA.—Peculiar to West Africa; found in the connective tissue; may occasionally be seen moving across the subconjunctiva. Embryos have a sheath, and appear in the blood in the daytime. Is non-pathogenic.

FILARIA PERSTANS.—West and Central Africa. Is non-pathogenic, and is found in the subpericardial, post-aortic and mesenteric connective tissue. Embryos in blood, no periodicity; the only filaria with a blunt tail. No sheath.

FILARIA DEMARQUAII.—West Indies. Male unknown. Non-pathogenic. Embryos in blood; no periodicity, no sheath, and a pointed tail.

FILARIA OZZARDI.—Demerara. Non-pathogenic. Embryos in blood; no periodicity, no sheath, and a pointed tail.

Filaria magalhaësi.—Rio de Janeiro. Recorded as found in a child's left ventricle. Life-history and pathology unknown.

Filaria philippinensis.—Manila. Adult unknown. Embryos in blood ; no periodicity, tight-fitting sheath, and a pointed tail. Non-pathogenic.

CHAPTER III

PUS—INFLAMMATORY AND SUPPURATIVE PROCESSES —STAPHYLOCOCCI — STREPTOCOCCI — MICROCOCCUS TETRAGENUS — PYOCYANEUS — GONORRHŒA — LEPROSY—GLANDERS—ACTINOMYCOSIS—ANTHRAX

PUS is a thick or thin turbid fluid of alkaline reaction, grey to green-yellow in colour, and of high specific gravity. On standing in the cold, it separates into two layers, an upper transparent layer of lighter colour and a lower opaque or turbid stratum consisting of pus cells, and occasionally giant pus cells and fatty cells. Smear preparations, suitably stained with any of the ordinary dyes, will demonstrate micro-organisms, of different forms and sizes, often in large quantities. Recent observations have shown that some of these organisms produce *tryptic ferments*, which, so to speak, digest the surrounding tissues, causing softening and breaking down, and investigations are at present in progress to discover an antitryptic body to limit or prevent these changes occurring, since when a layer of liquefaction necrosis occurs, it forms an impassable barrier to the bactericidal properties of the blood-stream, and prevents it acting to its full degree on the micro-organisms, thus causing disease to spread in the affected area.

In 99 per cent. of cases of suppuration bacteria are present, although experiments have also shown that it may be caused by chemical or mechanical irritants as well. Suppurative conditions are known as local inflam-

matory processes. Pus is the result of positive *chemiotaxis*, and consists of a dense collection of polynuclear leucocytes (with some mononuclears). The leucocytes, as well as being hurried up to the attack (positive chemiotaxis), are also newly formed in great numbers. Leucocytosis is also demonstrable in the general circulation, and is an important diagnostic sign that suppuration is taking place in some part of the body. Histolysis, or breaking down of the tissue cells, is due to the toxins of living bacteria, aided by the action of the endotoxins released by the dead bacteria.

Termination of Suppuration.

1. Pus may be removed surgically, or it may evacuate itself by bursting (abscess), or it may recover by phagocytosis.

2. Pus may extend by various alternative routes:

(*a*) By lymphatics—*e.g.*, plague, chancre.

(*b*) By anatomical proximity along ducts, etc.

(*c*) By the blood, by being washed away loose in the blood-stream, or by being carried away by the leucocytes, or by spreading by embolism after the formation of a septic phlebitis, or by direct extension through the walls of a vein—*e.g.*, portal vein.

3. Suppuration may terminate as a general septicæmia, with or without pyæmia.

4. The pus may become encysted, and get shut off from doing further harm by the formation of a strong encysting capsule.

Local inflammatory processes may be divided into acute or chronic, or pyogenic and granulomatous processes (tubercle and leprosy, see pp. 78 and 57). In the acute form there is a polynuclear leucocytosis at the site of infection, and in the chronic form (*e.g.*, tubercle) mononuclear leucocytes are found at the site of inoculation. The polynuclear leucocytosis is enormous; it comes on very rapidly and within a very short time.

Three-quarters of all pus productions are caused by

staphylococci. Three main forms of these organisms occur pathologically: *Staphylococcus pyogenes aureus* (golden colour), *Staphylococcus pyogenes albus* (white), and *Staphylococcus pyogenes citreus* (canary-yellow colour). These three forms cannot be absolutely distinguished from each other, but we may take it that they are different species.

True diplococci and streptococci chain-forms are often found as well.

STAPHYLOCOCCUS PYOGENES AUREUS, ALBUS, AND CITREUS, are so called from the colours of their growth on solid media. Although their colours are constant on such media, it should be noted that they are only varieties of one type, and vary under certain conditions. The individual cocci are colourless and spherical, about 1 μ in diameter, growing in clusters, due to their division in different planes. In young cultures diplococcic forms may also be seen.

STAPHYLOCOCCUS PYOGENES AUREUS.—This is the commonest of the three varieties, the most virulent, and the chief factor in acute inflammatory processes.

STAPHYLOCOCCUS CITREUS is the rarest.

Cocci are very widely distributed; they are normally present on the skin, from which they may be isolated, and they are the chief danger in all surgical operations. Many other varieties of staphylococci also exist in the skin, especially noticeable being *Staphylococcus epidermiditis*, which is very like *S. pyogenes aureus*, but less virulent in inoculation on animals, and liquefies gelatine less slowly; it, however, gives surgical trouble by infecting stitches, etc., of operation wounds.

THE STAPHYLOCOCCUS GROUP OF ORGANISMS.

Size.—They vary in measurement according to their culture media. In pus or discharges their size is fairly constant—1 μ.

Motility.—Non-motile.

Staining Reaction.—Stain readily with all dyes, and are Gram-positive.

Growth.—Grow readily in all the ordinary media; on *agar* they grow less abundantly, but with their characteristic colours; in *gelatine plates* grow and increase rapidly; smooth and glistening in appearance. In *gelatine stabs* liquefaction occurs about the fourth to fifth day, and as it proceeds the growth falls to the bottom as a coloured deposit.

On *potato* we get the characteristic colours. They render the various media acid, and coagulate milk. *Staphylococcus pyogenes aureus* a facultative anaerobe.

Spores.—They do not form spores.

STAPHYLOCOCCUS CEREUS (ALBUS AND FLAVUS) cannot be told from *S. aureus* by ordinary means, but in culture they produce a waxy growth; hence the name 'cereus.' They may become pathogenic at times.

Habitat.—Omnipresent in air, water and on the skin, and often on the healthy mucous membranes.

Vitality.—This is a point of the utmost importance to know in the case of all pathogenic germs, as it allows of prophylactic measures being undertaken. The staphylococcic group is very resistant to all bactericidal means, being the most resistant of all non-sporing organisms. A temperature of 60° to 65° C. will destroy most forms of bacteria as distinguished from spores, but it will not affect the *Staphylococcus pyogenes* group, as they will stand a temperature up to 70° C. When dry they will resist the temperature of boiling water for a short time. The importance of this is manifest in the case of the proper sterilization of surgical instruments used for operation or after operation on septic cases, as they should not merely be plunged into boiling water, but should be kept in boiling water for at least half an hour. Staphylococci, when dried in albuminous material, are also most resistant.

Virulence.—Very variable. It does not follow that staphylococci from a severe case are very virulent, and *vice versa.*

Pathological Conditions. — Staphylococci generally cause superficial suppurative conditions, acne furuncula (boils), sycosis, acute inflammations of joints and serous cavities. As a rule, they cause superficial inflammations, which may be called 'surgical inflammations,' in contradistinction to the deep-seated or 'medical inflammations,' which are generally due to streptococci and rarely to staphylococci. Septicæmia due to staphylococci may follow a local staphylococcic infection. Inflammations of the periosteum, bone, and of the cerebro-spinal system, ulcerative endocarditis, etc., may all be caused by staphylococcus infection. How the infection gets to these deep structures is unknown.

Experimental Inoculation.—This in the human subject has been proved by rubbing a culture on the skin of the forearm, whereby a carbuncular condition is produced. In animals lesions can be produced experimentally with results according to dosage and methods of infection.

Vaccine Therapy.—This is a most powerful and efficient remedy for chronic staphylococcic infections, particularly those causing sycosis and furunculosis. Sir Almroth Wright, while working at Netley, first carried out experiments which showed that the phagocytic power of the blood in these conditions was low, and he thought that if he could stimulate polynuclear leucocytosis it would be beneficial. A culture of *Staphylococcus aureus* (employment of the patient's own particular strain best) was made, and completely sterilized to kill all bacteria, and this was inoculated into the patient hypodermically. Results were encouraging, and further successes have since been obtained. The phagocytosis is increased in all cases, and the cure is permanent. The inoculations produce no fever and only slight temporary pain, the dose being 0·25 of a cubic centimetre of a twenty-four-hour

broth culture, repeated at intervals of a week. Sir Almroth Wright's results were obtained in chronic cases of inflammatory processes—*i.e.*, cases where there is a tendency to recurrence, such as sycosis and liability to boils, in which cases local treatment often proves unsatisfactory. In all chronic cases the phagocytic power of the blood should be frequently tested.

The culture employed may also be grown on agar and emulsified in water or normal salt solution. It should be sterilized by heat, using the lowest temperature possible to kill organisms (60° C. may be tried), and testing that they have been killed. The vaccine is next standardized by counting the number of cocci in a fixed small quantity, and then a small amount of antiseptic (lysol 2 per cent.) is added. The dose should be high (4,000,000 to 5,000,000 cocci) and should be repeated until the patient's phagocytic power is raised above that of a normal man. Staphylococci anti-sera (from an immunized horse) and anti-vaccine have been tried without good results.

THE STREPTOCOCCUS GROUP OF ORGANISMS.

An infinite number of varieties of these organisms exist both in health and in disease. Their differences on culture may be very slight, many being lost after subculture; but three main conditions are associated with the occurrence of *Streptococcus pyogenes aureus* in the human subject—namely, erysipelas, scarlet fever and puerperal fever. Streptococci, when experimentally inoculated in man, cause an 'erysipelatous inflammation.'

Morphology.—The individual cocci are indistinguishable from staphylococci, but are said to be slightly larger, and in fission there is a difference. In streptococci fission only takes place in one plane, forming chains, the length of which chains vary in different strains. In staphylococci fission may occur in any plane. In some cases,

however, staphylococci, diplococci, plague, and organisms from soft chancre (Ducrey's bacillus), may also grow in chains; therefore every chain one meets with does not necessarily prove a streptococcic infection, and the question can only be settled by inoculating animals, when, if an erysipelatous inflammation is not produced, the cocci are not the *Streptococcus pyogenes aureus*. The length of the chains produced by different organisms varies, and on this ground some observers have attempted to classify different varieties (longus and brevis); but, as the length of the chains depends entirely on the nature of the media on which they have been grown, this classification is not a good one.

Cultivation.—In media, especially solid media, streptococci are slow-growing (twenty-four hours), growing more slowly than staphylococci, and are in every respect more delicate organisms. On *agar* a small semi-transparent growth is seen; short chains only are seen, as they are liable to be broken on cultivation. They do not liquefy *gelatine*, whereas staphylococci liquefy it about the fifth day. In *broth* great variations occur. Fresh strains give long chains; the length depends, however, on the reaction of the broth. If slightly acid with tartaric acid, long chains are seen and the broth remains clear, except at the sides of the tube and at the bottom. The turbid appearance of the bottom is due to the long chains getting twisted into pellets, which stick on the sides of the tube or sink to the bottom. The broth afterwards becomes clear, with a cotton-wool-like sediment at the bottom. These changes in broth may distinguish *Streptococcus pyogenes aureus* from water streptococci and non-pyogenic cocci. The streptococci from scarlet-fever throats are called *S. conglomeratus*, from the tight balls they form in liquid media, and it is possible they are specific organisms. Streptococci are *facultative anaerobes*. They are best grown in blood-agar or inspissated serum, and, to get pure cultures, they are best grown on nutrient

broth and blood-serum. Erysipelas, scarlet fever and puerperal fever have special forms of streptococci for each. A scarlet-fever streptococcus *can* produce erysipelas, but *usually* it can only cause scarlet fever, and the same factor is true of the others. The fermentative reactions of streptococci are not of much importance.

Staining.—Streptococci stain by all ordinary means and are Gram-positive; many of the streptococci found in water are Gram-negative.

Virulence.—This influences the disease produced to a great extent, and is variable, especially if the streptococci are grown artificially, when they lose their virulence after many cultivations; but this can be restored by passage through animals—*e.g.*, a series of rabbits or guinea-pigs—from one to another, whereby it may be so increased that one single coccus can be made sufficient to kill a rabbit. Alternating growth between animals and special media will also increase virulence.

Pathological Conditions.—Streptococci are met with in deep-seated suppurative conditions (medical), as differentiated from superficial suppurative conditions (surgical), the latter being associated with staphylococci. Pus met with in acute inflammations of serous cavities and of the gastro-intestinal tract, etc., are generally caused by streptococci; but they may also be met with in other suppurative conditions, and, when found, these conditions are generally of a much more dangerous nature than when caused by staphylococci. In most streptococcic infections the organisms are not found alone, but associated with some other. This is termed *symbiosis*, and the resulting disease is much more grave, as they reinforce each other in their ill-effects. Examples of symbiosis are found in the case of diphtheria, and a prognosis can often be afforded as to the gravity of the infection by the presence or otherwise of streptococci with the diphtheria bacillus in swabs sent for examination. Some observers state that the *B. diphtheriæ* will not cause diphtheria unless the 'soil' (throat) has been prepared

by streptococci; the secondary ulcerations of small-pox, and the association of streptococci with the pneumococcus and the bacillus of influenza, will also afford a graver prognosis than when they are absent. Streptococci do not produce soluble *toxins*. The trouble in the host is probably due to endotoxins.

Vitality.—Streptococci are killed at 65° C., but are more resistant to high temperatures when exposed in the dry state.

ANTI-STREPTOCOCCUS SERUM.—This has been disappointing, and, not being much used clinically, it may be looked upon as a failure. It has been successful in laboratory experiments on horses. Failure in man appears to be dependent on the *numerous* multiplicity of strains of streptococci that may cause the infection. Polyvalent sera, prepared from a number of strains (twenty to thirty strains), with a view to hitting off the strain causing the disease in a particular patient, have been prepared from horses without any marked success. Monovalent strains for erysipelas, scarlet fever, etc., respectively, are more hopeful; but the line to follow is apparently to isolate the strain from the individual, and make the vaccine from this and treat with it, observing the opsonic index carefully and giving a dose equal to a hundredth part of the original dose, of about ten to fifteen million cocci.

Toxins of Streptococci and Staphylococci.—Even in local inflammatory processes the toxins are the chief cause of damage to the tissues, producing local necrosis (local tissue death). Mechanically the organisms do no harm by themselves, but the toxins, being entirely bound up in their bodies, can only diffuse when the organisms are no longer living. This fact can be well demonstrated by filtering a culture, when the filtrate is found to be innocuous.

Micrococcus Tetragenus.

This is also a pyogenic organism, chiefly found in the air, soil, dust, and also in the saliva of healthy individuals,

tubercular cavities of the lungs, and in various forms of suppuration. It multiplies by division in two planes at right angles. It has a capsule when found in the tissues. The capsule is difficult to demonstrate and is lost in artificial cultures. It is also met with in single forms and as a diplococcus. The individual cocci are indistinguishable from individual staphylococci and streptococci, and stain identical to these.

Cultivation. — Grows well in most media ; does not liquefy *gelatine.* The growth on all media is viscid and tenacious in character.

Staining.—Stains with all ordinary stains, and is Gram-positive.

Experimental Inoculation.—In white mice, which are very susceptible, it produces a general septicæmia, as well as an inflammatory local reaction.

Pathological Occurrence.—Comparatively rare, but an undoubted causal agent of pus, when it is generally found in association with streptococci or staphylococci ; it occurs also in symbiosis with the tubercle bacillus.

Bacillus Pyocyaneus.

This organism is the bacillus of blue pus, rarely seen in modern surgery. It is a short motile rod, not a coccus, 1·5 to 3 μ long, and not unlike *B. typhosus* in appearance. It is motile and has flagella. Does not form spores.

Cultivation.—Grows on all ordinary media, with formation of green pigment (fluorescence) ; liquefies gelatine very quickly, with green fluorescence. The colouring matter (pyocyanase) can be extracted and can be used to kill other bacteria (diphtheria) with success, as its local bactericidal effects are very powerful.

Staining.—All ordinary stains, but is Gram-negative.

Pathological Conditions.—Found in middle-ear disease (supposed to get in by the Eustachian tube) and, when found, the prognosis may be generally made of a very chronic course.

Vaccine.—Pyocyaneus vaccine is very successful. The immunity produced is that of an opsonic immunity—*i.e.*, increased phagocytic activity.

Other Pus-producing Organisms.

The more important of these are the diplococcus of pneumonia and gonorrhœa, the meningococcus, *B. coli communis*, *B. typhosus* (post-typhoid abscess, etc.), and the micrococcus of Malta fever. A host of unknown anaerobic organisms may also cause suppuration in connection with the intestinal canal (especially around the appendix).

Channels by which Local Inflammatory Processes may extend.

1. By lymphatics to glands, joints and serous cavities (pleura and peritoneum).
2. By natural channels—*e.g.*, urethra, bile-duct, etc.
3. By blood-stream directly, or indirectly as in septic phlebitis, the growth spreading along the walls of the vein—*e.g.*, portal vein.

GONORRHŒA—THE GONOCOCCUS.

The gonococcus was first described by Neisser in 1879. It is a capsulated diplococcus of kidney shape. The capsule is not so easily demonstrated as in the case of the pneumococcus. It is intracellular, being found chiefly inside leucocytes, and invariably occurs during the acute stages of gonorrhœa in the urethral discharges, and in gonorrhœal ophthalmia, and in all the complications of gonorrhœa.

Cultivation.—Very difficult to grow and to keep growing. Strictly aerobic, and very sensitive to changes of temperature. Special media are necessary for its cultivation, the best being Wertheim's medium (human serum one part, mixed with two parts of a 2 per cent. agar).

It will also grow on solidified blood-media, serum, agar, etc. It is interesting to note that Captain Potter, R.A.M.C., has found that the sterile blood of patients who have suffered from gonorrhœa, if smeared on agar, etc., gives a more luxuriant growth when the medium is inoculated than is the case when other blood is employed. On culture the gonococcus shows a remarkable tendency to undergo degeneration, becoming swollen and of various sizes (involution) losing its kidney shape and becoming round or oval (poly- or pleomorphous).

Staining.—It stains with basic aniline dyes, and is Gram-negative. In making a diagnosis by staining a smear preparation, it is of the utmost importance to use the Gram-staining reaction at the same time, and get its negative reaction, thus eliminating other diplococci, which may be mistaken for the gonococcus; then stain with weak carbol-fuchsin which stain the gonococci. By this method other pyogenic cocci are stained by Gram's stain, and the gonococci are stained red with the carbol-fuchsin.

Inoculation.—Inoculation experiments fail to produce the disease in the lower animals. Being very sensitive to changes of temperature, all inoculations must be made beside the patient and not at a later date.

Pathological Conditions.—The gonococcus is best found in the early stages of acute gonorrhœa, when lying in the leucocytes and in the superficial layers of the mucous membrane. In the later stages (gleet) they have penetrated deeper, and have worked back from the membranous urethra to the prostatic region, and few gonococci are to be found in the discharge. Occasionally they pass into the general circulation and give rise to a general septicæmia. The writer has seen two such cases which ended fatally. In both of these septic thrombosis of the prostatic plexus of veins occurred, and emboli containing the gonococci were found in many parts of the body. The disease may spread by the circulation and again localize

itself in a joint or joints and cause gonorrhœal rheumatism. In the testicle it may spread by direct extension along the vas, and cause epididymitis.

If a number of gonococci are found *outside* the leucocytes in the discharge of an early case, the disease is likely to run a severe or long course. If only a few are found outside these cells and phagocytosis is active, the case is more likely to run a mild course. In severe complications the organism is associated with other pyogenic germs (symbiosis)—*e.g.*, *B. coli communis*, staphylococci, and streptococci—and these may extend to the bladder, kidney, etc., or in the female to the uterus, Fallopian tubes, and peritoneum, causing severe lesions. Gonorrhœa in the female is generally found, not in the vagina, but in the cervix uteri. Gonococci have been found in the pleural cavity and a gonorrhœal endocarditis may also occur.

Method of examining a pus film containing gonococci :

1. Take some urethral discharge, as fresh as possible, and *lightly* spread it on a slide with a moist camel's-hair brush. If roughly treated, the leucocytes get ruptured, and the gonococci get out of the cells.
2. Fix in corrosive sublimate solution.
3. Stain by Gram's method.
4. Counterstain in carbol-fuchsin (1 in 10) for thirty seconds.
5. Result : nuclei of leucocytes and gonococci are stained red and Gram-positive cocci are stained dark.

LEPROSY.

This is a chronic specific granuloma of somewhat the same character as tubercle. It has a very widespread geographical distribution, and always presents the same general features in showing considerable tissue changes, with little impairment of the general health—*i.e.*, the local effects of the bacilli are well marked, but the toxic phenomena are at a minimum.

The Bacillus of Leprosy.—Discovered by Hansen in 1871; the bacillus is a short, thin, non-motile rod, about the same size as the tubercle bacillus, which it resembles in its straight or slightly curved appearance, and its acid-fast staining character (its ends, however, are pointed, while those of tubercle are generally rounded), and in being slightly thinner and rather more beaded than tubercle, so that they appear like rows of cocci. They have also a tendency to collect in clumps in the large mononuclear leucocytes. In the bacilli small spheres have been noticed and have been described as spores, but the evidence that they are true spores is not conclusive.

Staining.—An acid-fast bacillus staining more readily than tubercle by the Ziehl-Neelsen process with carbol-fuchsin and more easily decolorized, more dilute acid (5 per cent. H_2SO_4) for differentiation being preferable; it also stains by Gram, which brings out its beaded appearance well. Young bacilli stain more readily than what are supposed to be older ones, which do not retain aniline dyes well. Like tubercle, its fatty envelope can be demonstrated by osmic acid.

Cultivation.—All attempts to cultivate on artificial media or to inoculate animals with leprosy have failed. C. Nicholle is reported to have recently succeeded in inoculating subcutaneously the Chinese bonnet monkey (*Macacus sinicus*) with the juice of active leprosy tubercles, and has produced indurated nodules, showing an accumulation of lymphocytes and containing leprosy bacilli—in less number, it is true, than the human lesions—but containing no giant cells or caseation. The incubation period was from seventy-two to ninety-four days. The nodules remained without further change for two or three months, and then disappeared. It was observed that these monkeys became slightly immune to further inoculations, that the incubation period was retarded, and that, notwithstanding the susceptibility of monkeys

in captivity to tuberculosis, none of those inoculated with leprosy developed tubercle.

Microscopic Diagnosis. — 1. Films made from the discharge of ulcerated nodules, or from scrapings stained by the Ziehl-Neelsen method, will show large numbers of bacilli lying in the cells.

2. Films made from swabs from the nasal secretion are of the utmost importance in cases of suspected leprosy, which is often (70 per cent.) primarily a disease of the nasal septum. In the nasal mucus of 153 cases examined by Sticker he found bacilli in 128; other observers, by provoking an experimental coryza, have caused the bacilli to appear.

3. In microscopic examination of sections from excised pieces of granulomata, etc., the enormous quantities of bacilli, when stained by the Ziehl-Neelsen method, is almost pathognomonic of the disease.

VARIETIES OF LEPROSY.—There are three chief forms: (1) *Lepra tuberosa* (*tuberculosa*), or nodular leprosy; (2) *Lepra anæsthetica*, or nerve leprosy; (3) the mixed type, or a combination of these, is really the most common.

1. *The Tubercular Form.*—The disease starts as erythematous patches, which may subside, to appear again later on, followed by the development of nodular thickenings, leading to ulceration of the skin, especially of the face and eyebrows (leonine type), extensor aspects of arms and legs, back of hands and feet, which may enlarge and produce great distortions. The mucous membranes of the eye, mouth, larynx and pharynx may also be affected. High fever may occur at irregular intervals, and during attacks the bacilli get into the blood and internal organs; spleen, liver and testicles may become secondarily affected. The change occurs chiefly in the true skin and is of a chronic inflammatory character, containing 'lepra cells,' which are large mononuclear leucocytes full of bacilli. Some think these are

thrombi in lymph channels, but the remains of a nucleus in the centre of the clump can often be seen. They are found in the granulation tissue wherever it occurs, and in this there is a varying amount of stroma, bloodvessels and perhaps atrophied nerve-endings. Caseation does not occur.

2. *The Anæsthetic Form.*—The initial lesion is chiefly in the sensory rather than the motor nerves. These undergo diffuse infiltration, causing destruction of their fibres, but there are not so many bacilli as in the tubercular form. In the early stages there may be pain along the nerves, followed later by paralysis and atrophy of muscles, atrophy of bones, causing distortion, loss of sensation and various other trophic disturbances. The skin may undergo atrophy and appear like shiny parchment. Owing to injury, to which the anæsthetic parts are liable, necrosis and separation of fingers or toes are likely to occur. Blindness may also happen, due to paralysis of the ocular muscles and conjunctiva, dirt getting into the eye. In fact, any variety of change is met with in this form of leprosy.

INFECTIBILITY, DURATION, PROPHYLAXIS, ETC.—There is considerable difference of opinion as to the infectibility of leprosy. Recent observations tend to attribute the spread of the disease in the first instance to insects. *Acarus scabiei*, the bed-bug (*Cimex lectalarius*), and the mosquito (*Culex pungens*), have both been found infected with bacilli. In support of the inoculation theory, the case of a criminal in the Sandwich Islands who was experimentally infected is quoted; while the contact or contagious theory is supported by the case of a man in Dublin who was supposed to have contracted the disease from his brother, also that of Father Damien, who became infected from lepers in the Sandwich Islands. Leprosy appears transmissible, but healthy children have been born to leprous parents. The incubation period is long—two years or more—and the duration of the disease

is chronic. The tubercular form may last nine years, the nervous eighteen, and the results are invariably fatal. Death, however, is often due to intercurrent diseases, chiefly tubercle; and when this occurs, the tubercle bacilli have their usual characters, showing there is no connection between the two diseases. The resistance of the bacillus is unknown, as it cannot be cultivated outside the body. Segregation prevents the spread of the disease, as has been demonstrated in Norway, Sweden, etc. Recently 'Nastin' (a neutral fat made from *Streptothrix leproides*, isolated by incubation in normal salt solution at 37° C. for several weeks, and derived from excised tissue from non-ulcerated lepromata, treated with 2 per cent. benzoyl-chloride, forming 'benzoyl-nastin') has been used by Deycke as a curative treatment. It is supposed to act directly on the leprosy bacilli, the benzoyl removing their fat and the nastin producing an antibacterial effect.

GLANDERS.

This is a highly infectious specific disease affecting chiefly horses, mules, and asses, sometimes other domestic animals, and occasionally transmitted to man by accidental inoculation. Horned cattle are immune, goats and sheep tolerant, and carnivora may become infected by eating raw glandered meat. Grooms, stablemen, muleteers, and others in contact with equines, are most liable to become infected. So highly infectious is glanders that it is inadvisable to make a post-mortem examination.

THE BACILLUS MALLEI.—Minute straight or slightly curved non-motile rods, with rounded ends, varying in size, but generally of about the same size as tubercle bacilli, but thicker; may show a beaded appearance. Debatable as to whether they contain spores, but their low power of resistance is against this.

Propagation, etc.—In animals early recognition of the disease is of utmost importance, from its great infecti-

bility. In the acute form there is fever, with nodular, grey, translucent swellings of the nasal mucous membrane, chiefly that of the septum nasi; and profuse nasal catarrh. The latter is highly infectious, and may infect the water-supplies and drinking-troughs of animals. Similar lesions also occur in the respiratory system, also nodules in the lungs, spleen, liver, lymphatic glands, etc. These vary from the size of a pea, or larger, of yellow or grey colour, congested periphery, and firm or softened centre. When infection takes place on the skin, from harness rubbing, etc., the superficial lymph glands enlarge and ulcerate (*farcy buds*) ; the lymphatics enlarge and thicken, and appear as knotted cords (*farcy pipes*).

In the chronic or latent form the disease may only be detected by injecting mallein, and getting a rise of temperature over 1·5° C., or by the agglutination of glanders bacilli with a 1 in 50 dilution of the animal's serum. The disease may be transmitted to man by direct inoculation of cuts or abrasions, either in grooming an infected animal or skinning a dead one, or by means of nasal mucus sneezed into the eye, nose, or mouth, or by a bite. In man the *acute* form begins as a boil or ulcer at the site of inoculation (hands or forearms), constitutional symptoms, quickly followed by papular eruptions, which become pustular, intramuscular abscesses, joint suppurations, pyæmia, coma and death within fourteen days. In the *chronic* form the local lesions predominate ; chronic ulceration is followed by induration and lymphangitis, metastatic abscesses, pulmonary infection, with hæmoptysis, coma and death in 50 per cent. of cases.

METHODS OF EXAMINATION.

1. *Microscopic examination* of films from nasal mucus or pus will show the *Bacillus mallei*, but an absolute diagnosis cannot be made without culture, inoculation of a guinea-pig, and other tests.

2. *Staining.*—Stain easily with all dyes and are Gram-negative. On *potato* at 37° C. on third day a charac-

teristic growth—yellow, transparent, and like honey—at the margins of which the potato stains greenish-yellow.

3. *Inoculation* of a male guinea-pig (intraperitoneal) within three days causes acute purulent inflammation of the tunica vaginalis and orchitis (Strauss's reaction).

4. *Mallein Reaction.*—Of great value, but only applied to suspected animals, not man. Infection of mallein in a positive case causes a rise of temperature of over 1·5° C.

5. *Agglutination Reaction.*—The *Bacillus mallei* is agglutinated in a 1 in 50 dilution by the serum of an infected animal. This is not, however, an absolutely reliable test.

ACTINOMYCOSIS (*Discomyces bovis*).

ACTINOMYCOSIS, or the *ray fungus*, discovered by Bolinger in an ox in 1877 and by Israel in man in 1878, is chiefly a disease of oxen, horses, and swine; less frequently it affects man. The parasite is not a true bacillus, but a streptothrix, and as other species of this group affect man, the correct nomenclature of the disease, according to Sir Patrick Manson, is actinomycotic mycetoma, and the streptothrix is *Discomyces bovis*, in contradistinction to some half-dozen other tropical streptothrix diseases, the chief of which is known as 'Vincent's white mycetoma,' or 'Madura foot,' caused by *Discomyces maduræ*.

Actinomycosis in cattle leads to the development of large tumours in the tongue ('woody tongue'), jaw, floor of mouth, neck, etc., which ulcerate; in man it chiefly affects the mouth, and gives rise to chronic inflammation and suppuration.

MICROSCOPIC DIAGNOSIS, ETC.—This can be made with certainty by the detection of the characteristic granules in the secretions, whether pus, sputum or urine. The pus is thin, viscid and stringy, with yellow or dark grey granules visible to the naked eye; micro-

scop'cally, these appear as closely packed spherules, formed by radially arranged club-shaped bodies (clubs) around the periphery, which taper off towards the centre of the granule, and then form a dense branched network (mycelium) of fibres, within which spherical bodies (supposed to represent the spores or gonidia of artificial cultures) may be seen.

Staining.—The granules should be broken up by gentle crushing on a slide, and after fixing, stained by Gram and counterstained with weak carbol-fuchsin. The filaments and spores are Gram-positive and the clubs usually Gram-negative. In some cases this streptothrix is 'acid-fast.'

Cultivation.—Glycerine agar at 37° C. after three days shows a yellowish-red, elevated, nodular, tough, discrete growth, which adheres firmly to the medium and tends to become corrugated in time. In broth pellets form. On gelatine the growth is slow and the medium is slowly liquefied.

Experimental Inoculation.—Uncertain ; can, however, be inoculated in bovines ; rabbits and guinea-pigs not susceptible to all strains.

Sources of Infection.—Parasite grows on grain, especially barley, which infects animals or man when eaten raw through broken surface of buccal or other mucous membrane, crypts of tonsil, spongy gums around decayed teeth, etc. Swelling and suppuration are followed by metastatic abscesses, periostitis, and necrosis of bone. Respiratory and intestinal forms of the disease also occur in man. In the ox the disease is usually localized to the tongue (producing woody tongue) and tissues in the vicinity of the bucco-pharyngeal cavities, forming hard, tumour-like masses, which tend to ulcerate.

ANTHRAX.

This is a fatal epidemic specific septicæmia, caused by the *Bacillus anthracis*, found amongst herbivora, especially

sheep and oxen, and to a lesser extent amongst horses, mules and pigs, being occasionally transmitted to man from these animals, their carcasses, hides or hair, by inoculation, respiration, or ingestion, causing at first a local inflammation at the site of infection (skin, respiratory system, or intestine) which may terminate in a fatal septicæmia.

The Bacillus Anthracis.

Microscopic diagnosis of this organism is very easy.

1. *Hanging Drop.* — Large, non-motile, facultative anaerobic, spore-forming bacilli, 6 to 8 μ long, 1 to 2 μ thick, ends flat (sometimes round or cupped), lying end to end, often forming long threads with a thin bright capsule common to the whole thread. Spores, if present, appear as refractive specks in the centre of the bacilli.

2. *Staining.*—Is Gram-positive, but stains easily and deeply with all basic aniline dyes ; owing to density of these stains, spores are not visible, but can be demonstrated by special methods (see p. 143).

3. *Cultivation.*—After twenty-four hours on *agar* at 37° C., colonies under low power show characteristic wavy dentate margins, formed by long undulating hair-like chains of bacilli. In *gelatine* stab after forty-eight hours at 20° C., a growth like an inverted deodar fir-tree appears, the longer branches radiating from the stab (central stalk), being longest near the surface. Later liquefaction, beginning at surface, spreads downwards. In *broth* at 37° C. after twenty-four hours spiral threads form ; these, sinking later to the bottom, form a cotton-wool-like sediment, similar to that formed in broth by streptococci.

4. *Inoculation.*—Subcutaneous inoculation in guinea-pig causes gelatinous infiltration at site, followed by septicæmia and death. Bacilli are found in the blood, and in all organs, especially liver, spleen, heart.

5. *Resistance.*—If spore-free, have low power of re-

sistance; killed by gastric juice in health, putrefaction and 60° C. Optimum temperature for growth 35° C., minimum 12° C., and maximum 45° C. Sporulation requires oxygen, optimum temperature being 30° C., minimum 18° C., maximum 42° C.: thermal death-point boiling for five minutes, or 140° C. dry heat for several hours; can resist gastric juice and antiseptics probably longer than any other micro-organism. To prove spores, heat to 80° C. for an hour and inoculate media.

HUMAN ANTHRAX.—In man the disease is always derived either directly or indirectly from animals, their carcasses, hides, or hair, and may appear in either of four forms: (1) malignant pustule, (2) respiratory system, (3) intestinal, (4) septicæmia. The site of infection determines the variety. If through cuts, abrasions, or hair follicles, malignant pustule results; this occurs amongst workers in carcasses (butchers), hides, bones, and those looking after animals (herds, grooms, muleteers, etc.). The respiratory form, known as 'woolsorter's disease,' occurs amongst those inhaling spore-infected dust, hair or wool from hides, and those working in hair, bristle, and brush factories. The intestinal form takes place from ingestion of spores in food, as in the case of eating uncooked infected meat, and by animals eating grass infected by blood or discharges. The septicæmia form, which is rare, occurs without any apparent lesion.

MALIGNANT PUSTULE.—This is generally met with on the exposed surfaces—the face, hands, forearms or back, the last being a favourite position among hide-carriers. An officer infected a scratch on his neck from the horse-hair padding of a broken cushion in a first-class railway-carriage. Within three days of inoculation a painful pimple appears, becoming congested with a vesicle in the centre; central necrosis sets in, and a black eschar results, surrounded by œdema and lymphangitis, causing fever, with general malaise. Widespread œdema and

buboes follow. If not relieved by excision, toxæmia and death may occur, but as a rule few bacilli are found in the blood in man.

RESPIRATORY ANTHRAX, OR WOOLSORTER'S DISEASE.—The primary lesion exists either in the lower part of the trachea or larger bronchi, causing hæmorrhagic œdematous patches of the mucous membrane and enlargement of the mediastinal and bronchial glands, pleural and pericardial effusion, petechial hæmorrhages on the serous membranes, and perhaps œdema or collapse of lung tissue, followed by septicæmia. With the exception of the thoracic tissues and glands, at times it may be impossible to find bacilli, but, on the other hand, the characteristic rods may be found in the sputum.

INTESTINAL ANTHRAX.—In this rare form localized hæmorrhagic œdematous patches of the intestinal mucous membrane, with central necrosis and enlargement of the mesenteric glands, are found.

MICROSCOPIC DIAGNOSIS.—In suspected malignant pustule, film preparations from either the vesicular fluid or from puncture at the periphery of the eschar or neighbouring lymphatic glands will, on staining by Gram, show the typical rods, although these may sometimes be very scanty. No other condition, however, occurs in which they are present, with the exception of malignant œdema. Culture on *agar* incubated at 37° C. should be used to confirm, or subcutaneous inoculation of guinea-pig or mouse which causes death from anthrax septicæmia within two days.

PROPHYLAXIS.—Horses, cattle, or sheep, suffering from anthrax may, without any symptoms beyond being a little 'off colour,' suddenly drop dead from septicæmia, or in less acute cases show general malaise, with slight blood-discharge from the bowels, mouth, or nose.

Amongst horses and mules œdema and ulcerated carbuncles of the neck may be present. In nearly all cases in animals the bacilli are present in the blood just

before death, and blood taken from the ear, first shaving off the hair to avoid further infection, usually shows them in fair numbers. It should be remembered that as anthrax requires oxygen, it only spores outside the body, and not inside; once exposed to air, it spores readily, and the importance of not contaminating the hair round the site of the puncture made for diagnostic purposes with blood should not be overlooked. No *post-mortem* should be made on animals dying from anthrax, beyond blood examination. This is best done by plunging a long hypodermic needle or drawn-out glass tube into the heart, and drawing off blood. If earth or grass is contaminated by discharges or blood, these on drying cause the bacilli to sporulate, and grass has been known to be infective by such means for twenty-three years when eaten by other herbivora. Carcasses should be covered with quicklime and buried in a pit (not less than 4 feet deep), and all discharges, blood, etc., burnt.

TREATMENT.—Destroy local infection, and if 30 to 40 c.c. of anti-anthrax serum (Sclavo's) be injected in 10 c.c. doses before anthrax septicæmia sets in, it proves successful. Anthrax was the first disease for which vaccine was made, and it has been a very successful prophylactic for epidemics occurring amongst herbivora.

CHAPTER IV

THE BUCCAL AND NASAL SECRETIONS—THE SPUTUM—THEIR PATHOGENIC BACTERIA AND OTHER PARASITES—THE PNEUMONIAS—THE PNEUMOCOCCUS—THE PNEUMO-BACILLUS—EPIDEMIC CEREBRO-SPINAL MENINGITIS—LUMBAR PUNCTURE—TUBERCULOSIS—THE TUBERCLE BACILLUS

THE BUCCAL SECRETION.

THE mouth is never sterile. There are always present the bacteria constituting its indigenous flora which are not necessarily pathogenic; from time to time occur pyogenic organisms of the staphyococcus and streptococcus groups, and those which are concerned with the production of caries and diseases of the pulp of the teeth, the various varieties of *leptothrix spirochætæ*, and the *Bacillus gangrenæ pulpæ*, and lastly those of a more dangerous nature, such as the *pneumococcus*, *tubercle bacillus*, and many other pathogenic organisms. The mouth affords a most favourable medium, not only for the multiplication of bacteria, but also for the spread of sepsis to other organs and the absorption of toxins. If the mouth be rinsed out with a warm solution of bicarbonate of soda, followed by cold water, and the mucous membrane lightly touched with a glass rod, a fairly pure sample of saliva can be obtained. The reaction of saliva is alkaline. Salivary corpuscles resemble large leucocytes with a granular protoplasm. The chief fungi and organisms which occur are *Spirochæta buccalis*, and forms resembling the *Comma vibrio*,

Leptothrix buccalis, *Micrococcus tetragenus*, the bacillus of decaying teeth, *Staphylococcus pyogenes albus*, *Pyogenes aureus*, *Streptococcus pyogenes*, and the *Bacillus diphtheriæ*. All of these may exist in an innocuous state. Under pathological conditions the *tubercle bacillus*, the thrush fungus, and *actinomyces* may occur.

THE TONSILS.

A deposit on the tonsils may be due to *streptococci*, *staphylococci* or to the *diphtheria bacillus*, either in combination, alone, or almost alone. It must be remembered that even in the normal state streptococci and many of the organisms existing in the buccal cavity may also be found on the surface of the tonsil. Pathologically, the *bacilli of diphtheria* (and their pseudo-forms), *plague*, and *influenza*, are the more important.

Influenza Bacillus.—This organism often occurs in great numbers on the tonsil or in the sputum, in the early stages of the disease free, and later inside the pus cells. A small portion of the yellow purulent exudate should be selected, and smear preparations made. The bacilli are non-motile, straight, with rounded ends, $1{\cdot}5\ \mu$ long and $0{\cdot}3\ \mu$ broad ; sometimes polar staining is visible. They stain readily with Löffler's stain with the aid of heat, and with dilute Ziehl-Neelsen fluid, but not by Gram's method. The medium best for producing growth is blood-agar.

The pathological lesions produced and the processes recommended for the detection of the *Bacillus diphtheriæ* are described in Chapter VI., p. 101, and for the plague bacillus in Chapter V., p. 95.

THE NASAL SECRETION.

In suppurating conditions of the nasal cavities the nasal discharge may consist almost entirely of pus cells ; in tuberculous and leprous ulcers the specific organism may be looked for. In cases of glanders the presence of

the bacillus will determine diagnosis. The diphtheria organism occurs in some forms of rhinitis fibrinosa. The thrush fungus, ascarides, and other entozoa occasionally occur, and dipteral larvæ are not infrequent.

THE SPUTUM.

Of the non-pathogenic moulds, the *thrush fungus* may occur, derived from aphthous patches in the mouth; in rare cases it may, however, be derived from the bronchi in children. Of the non pathogenic fission fungi, mention must be made of *sarcinæ*, which are associated with extensive ulcerative or destructive conditions affecting the lung. A non pathogenic streptothrix (Leishman and Birt's) is of some importance, as it is acid-fast, staining by the Ziehl-Neelsen method, and small filaments may possibly be mistaken for the tubercle bacillus. It is, however, rather larger and not beaded, and some pieces show branching, with arthrospores at the ends. On agar the colonies are very hard and calcareous, and of a pinkish colour. Many forms of bacilli and micrococci occur in ordinary sputum, including the *Diplococcus pneumoniæ* and *Bacillus diphtheriæ* in a non-pathogenic state.

Pathogenic Organisms.—The more important of these are the *tubercle bacillus*, the *pneumococcus* and *pneumobacillus*, and the bacilli of *influenza* and *plague*, a pathogenic *streptothrix* and *actinomycosis*, certain *infusoria*, and vermes (ascarides, hooklets from echinococcus cysts, and eggs of *Distoma hæmatobium*).

Tubercle Bacillus.—This bacillus cannot be seen in unstained sputum. A note on its life-history and pathological significance is to be found on pp. 78 to 82.

MICROSCOPIC DIAGNOSIS.—Cheesy particles are selected for making film preparations, and should any difficulty be found in demonstrating the presence of the bacilli, the following methods may be tried: Mix the sputum with some 1 in 20 carbolic acid in water; let the mixture stand, and examine some of the sediment; or the

addition of a small amount of a 10 per cent. aqueous solution of caustic potash to the coagulate rapidly breaks up all caseous particles and digests the mucus; the residue is then shaken up, and let stand, some of the sediment being spread on a slide and stained by the Ziehl-Neelsen method.

Micro-organisms of Pneumonia.—These are fully discussed on pp. 71 to 74.

Diphtheria Bacillus.—This may occur in the sputum in severe cases of the disease, infection reaching the lungs from the blood; broncho-pneumonia may be present. The methods of microscopic diagnosis are discussed in Chapter VI., pp. 102 and 103.

Influenza Bacillus.—This organism often occurs in great numbers in the sputum, either in pus cells or lying free (see pp. 99 and 100).

Streptothrix.—A pathogenic variety of this organism has been recorded, which gives rise to primary lesions in the lungs of the nature of a suppurative pneumonia running a rapid and fatal course. The organism occurs as a Gram-positive acid-fast mycelium in the pus. In some cases pure cultures were readily obtained as a surface growth on various media, and typically on bouillon and potato. The organism exhibited a pleomorphism in cultures, the mycelium only appearing in very young cultures, whilst later three varieties occurred—*i.e.*, short-branching filaments, spore-like bodies, and short rods resembling bacilli. Experimentally it was pathogenic for guinea-pigs and rabbits, more especially for the former, the testicular enlargement which was produced in these being strongly suggestive of glanders inoculation experiments. The organism is probably the same as described by Eppinger and later by Stewart Macdonald, and experimentally these species have produced miliary nodules indistinguishable from the so-called histological tubercle. Tubercles of this sort occurred in the cases recorded, and in the giant-cells acid-fast bacilli were found, but no filaments of streptothrix, though

further experiments were made in the hope of producing those conditions.

Actinomyces.—The ray fungus occasionally infects the lungs, and the characteristic granular masses may be demonstrated in the sputum. For further details, see pp. 61 and 62.

Plague Bacillus.—As the sputa of patients suffering from plague, especially the pneumonic form, contain these bacilli often in large numbers, microscopic examination is of great importance. The oval forms of bacilli, showing bipolar staining, and the very characteristic degeneration forms, faintly stained, round or oval, of variable size, are almost pathognomonic. For a detailed description of plague, see Chapter V., pp. 95 to 99.

Infusoria.—Monadines and cerco-monadines, showing sluggish movements, may occur in gangrene of the lung. The organisms are described in Chapter VII., pp. 115 and 116.

Vermes.—Rarely *ascarides* and cysts, or hooklets of echinococcus and eggs of *Distoma hæmatobium*, occur in the sputum. These are described in Chapter II., p. 37.

THE PNEUMONIAS.

The pneumonias are of three main varieties: (*a*) croupous or lobar pneumonia, (*b*) catarrhal or lobular pneumonia, and (*c*) septic pneumonia. These three types are associated with different organisms.

Croupous Pneumonia.—This is an acute inflammatory process, attended by an abundant fibrinous exudation, affecting by continuity the entire tissue of a lobe or of a large portion of the lung or lungs. It departs from the course of an ordinary inflammation in that the reaction of the connective tissue of the lung is comparatively slight, and that there is usually no tendency for organization (into connective tissue) of the fibrinous exudation to take place, resolution occurring instead. Fibrin, red corpuscles, and leucocytes are found in the alveoli. Croupous

pneumonia is directly due to the pneumococcus in 95 per cent. of cases, and to the pneumo-bacillus in the remaining 5 per cent.

Catarrhal or Lobular Pneumonia.—In this a catarrhal inflammation spreads from the capillary bronchi to the alveoli. In the capillary bronchi there is a proliferation of the endothelium, which leads to small consolidated patches of lung tissue. Catarrhal pneumonia is found as a *primary* disease in children, and in adults as a *secondary* complication in influenza, Malta fever, diphtheria, enteric fever, plague, anthrax, etc., and is due to the presence of the respective organisms of these diseases in the lung. Damage to lung tissue rather than resolution may occur from the thickening of the connective tissue of the alveolar walls which accompanies the process.

Septic Pneumonia.—This may arise in two ways: (1) From discharges (operation wounds or otherwise) entering the trachea or bronchi; (2) from septic pyogenic infection (generally streptococci) entering the blood-stream from other parts of the body, and lodging as septic emboli in the lung capillaries.

THE PNEUMOCOCCUS.

Morphology.—The diplococcus of pneumonia, otherwise known as the 'diplococcus of Fraenkel-Weichselbaum' (the discoverers), or the 'diplococcus lanceolatus,' from its similarity to the pointed end of a lancet, is a capsulated, non-motile, and non-sporing organism. The capsule is lost in artificial cultivation. The organism is a Gram-positive one.

Cultivation.—Difficult to grow on ordinary media, but grows readily on blood-media (3 parts broth and 1 part ascitic fluid is also to be recommended), especially rabbit's blood-agar, which is said to maintain virulence. The latter is made by smearing rabbit's blood over an agar tube (Pfeiffer's medium). The growth is almost transparent, the pellicle along the centre with isolated colonies

at the margins. In a *gelatine* stab the growth appears as a row of minute points, which remain a small size, and there is no liquefaction. On plates the colonies are almost invisible, but under the microscope they have a finely granular centre, with a pale periphery. In culture the pneumococci frequently lose their shape, and may appear as isolated cocci or in chains. They do not form spores, and are non-motile.

Vitality.—The pneumococcus is easily killed by a temperature of 60° C., unless when in the dry state, so that infection may be caused by dry sputum for weeks. It is preferably an aerobe, but can exist without oxygen.

Isolation.—The rabbit, being very susceptible, is used for obtaining pure cultures by inoculation.

Virulence.—This is easily lost, and the best way to maintain it is by growth on rabbit's blood-agar.

Pathological Conditions.—The pneumococcus is found in healthy throats (one in every five cases), and from saliva it is very virulent when inoculated into the rabbit. In man the cocci may invade the tissues pathologically when vitality is low.

The pneumococcus is not confined to pneumonia; it may also occur in otitis media, suppurations, empyema (by extension), ulcerative endocarditis, cerebro-spinal meningitis, conjunctivitis, and any of these conditions, including pneumonia, may pass into a pneumococcus septicæmia.

Bacteriological Diagnosis.—This can be made by—

1. Direct examination of smears made from rusty sputum.
2. Experimental inoculation of a patient's rusty sputum into a rabbit. If the pneumococcus is present it causes a general septicæmia, and the rabbit dies in forty-eight hours. Rabbits and mice are very susceptible; guinea-pigs, rats, and dogs are intermediate; and the pigeon is immune, probably because of its higher blood temperature (104° F.).
3. Culture on media (already described).

THE PNEUMO-BACILLUS OF FRIEDLANDER.

It is not clear that this organism is the cause of pneumonia, but it is found in about 5 per cent. of cases. It somewhat resembles the pneumococcus, but is broader, and it occurs in short or long rods in culture, tending to grow in pairs, and has a capsule which is not seen in culture. Involution forms are common.

Staining.—Stains with basic aniline dyes, and is Gram-negative.

Cultivation.—Grows in ordinary media and on gelatine plates. The colonies are white raised discs, and appear like small ivory knobs. Gelatine stab gives a characteristic white 'round-headed nail' growth, with no liquefaction, but occasionally bubbles of gas appear. In culture longer rods are formed than appear in the body. Poly- or pleomorphism, or changes in shape and size, also occur.

Inoculation.—Pathogenic to mice and guinea-pigs, but not to rabbits.

EPIDEMIC CEREBRO-SPINAL MENINGITIS.

The causal agent of this disease is the *Diplococcus intracellularis meningitidis*, or *Micrococcus meningitidis cerebro-spinalis*, first described by Weichselbaum in 1887. Other forms of cerebro-spinal meningitis occur associated with other organisms, more particularly streptococci and pneumococci. Epidemic cerebro-spinal fever attacks young people, chiefly children and young adults (young recruits), and has a high mortality, which may reach 70 per cent. of cases. Its epidemiology is not clear. Mysterious cases first crop up, having no apparent connection with each other; it may then become epidemic, and is of particular importance to army surgeons, as severe fatal epidemics have occurred in the French and German armies, as well as small, less severe

outbreaks in our own, and isolated cases have occurred amongst the garrisons at Belfast, Dublin, and Aldershot within the last few years. The disease may run either an acute course, proving fatal in a few days, or a subacute course, when it is not so fatal, or it may run a chronic course, which is very fatal. The chief clinical features of the disease are: (*a*) It attacks apparently healthy people; (*b*) sudden prostration, with high fever, headache, and vomiting; (*c*) stiff neck; (*d*) convulsions and paralysis (rarer); (*e*) purpuric rash (hence its name, 'spotted fever'); (*f*) blood changes—(1) marked leucocytosis, ranging from 13,000 to 62,000 per cubic centimetre, polymorphs in excess, diminution of small lymphocytes, and absence of eosinophiles; (2) a high red count, high percentage of hæmoglobin, and a high colour index; (3) negative glycogenic reaction.

The meningococcus is found in the pus cells of the flocculent exudate, which forms green pus in the membranes of the brain and spinal cord and in the ventricles of the brain. Intestinal lesions may also occur as patches of reddening, or even ulcers and enlarged mesenteric glands, from which the organism has been stated to have been isolated. In cases that recover agglutinins are found in the blood.

Two varieties of the disease are described: (*a*) the fulminating type; (*b*) the typhoid type.

Microscopic Diagnosis.—The meningococcus is a rounded or oval diplococcus, with flat surfaces opposing, resembling the gonococcus, and like it in its intracellular character, being found inside leucocytes; but it differs from it in appearance, having its opposing surfaces flattened and not kidney-shaped. Tetrads may occur. It is capsulated, but the capsule is hard to demonstrate.

Staining.—Does not stain by Gram; this distinguishes it from the diplococcus of pneumonia. The Gram reaction is, however, not constant.

Culture.—It is easier to cultivate than the gonococcus

but requires special media, blood-serum on agar, etc.; on these it grows readily, forming transparent colonies. No growth on potato. Is a strict aerobe. Tends to die out in subculture. Maltose, galactose, and dextrose are fermented. Is non-motile. Does not form spores. Agglutination occurs late in the disease (ten to twelve days).

Vitality.—Slight outside the body; easily killed by heat and antiseptics, and not very resistant in the dry state.

Inoculation.—Animals when inoculated do not afford much information.

Relation to Disease.—The meningococcus being found in the nasal mucus suggests an entry from air, but it has never been found in the air. It is supposed to reach the cerebrum through the naso-pharynx by the cribriform plate of the ethmoid, or it may get into the system by the intestinal lesions, which are suggestive. Other diplococci found in nasal mucus have to be differentiated from the meningococcus; the chief of these are the diplococcus of Crassus, which is Gram-positive, and the *Micrococcus catarrhalis,* met with in diplococcus form, which is Gram-negative, but more opaque and sticky to the needle when grown on agar. Fermentation process on sugars will differentiate these; the latter organism does not ferment sugars.

Prophylaxis.—Immediate notification; strict isolation; segregation of contacts; syringe nose and naso-pharynx of all contacts with weak formalin; disinfect rooms and clothing.

LUMBAR PUNCTURE AS AN AID TO DIAGNOSIS.

Valuable information as an aid to diagnosis can sometimes be obtained in cases of spinal and cerebral diseases, especially meningitis, by lumbar puncture. There are

many methods recommended for performing the operation, but the following gives good results: The patient is made to lie on his right side, with the pelvis slightly raised by a firm cushion (such as is used on operation-tables), and is told not to move when he feels the sharp prick of the needle. After sterilizing the skin, the sterile needle of a large syringe, such as an antitoxin syringe, is inserted in the middle line and passed forwards and slightly upwards between the spines of the third and fourth lumbar vertebræ, which lie in the same transverse plane as the highest point of the iliac crests. When the spinal canal is entered (the distance of this from the surface is variable, according to muscular development, etc.; in the child it is about 2 cm. deep, and in the adult 4 to 6 cm.), the cerebro-spinal fluid will come out in drops. About 5 c.c. of this should be caught in a sterile test tube,* the needle quickly withdrawn, and the wound sealed with acetone collodion. Lumbar puncture was first practised in England by Dr. W. Essex Wynter in 1889. Since that time the procedure has fully established its position in the armamentarium of the modern clinician. It is an operation entirely devoid of risk if aseptic precautions are adopted, and will not only afford valuable data in the diagnosis of many diseases, but also in certain cases it has a very decided therapeutic value. The pressure of the cerebro-spinal fluid varies greatly in normal conditions. In healthy persons in the horizontal position it averages 125 mm. water-pressure, but in the sitting posture this might increase to more than 400 mm. In pathological conditions it is sometimes impossible to determine how far a reading will give an index of the pressure of the fluid as a whole or only of a part shut off from the rest by adhesions. Normal fluid contains globulin, and the pres-

* The cerebro-spinal fluid thus obtained should be centrifuged, any deposit or sediment collected, smear preparations made and examined for organisms, or inoculations made from it in animals.

ence in it of albumin is evidence of organic disease. The same might be said, too, of choline, but the tests for choline are difficult to carry out clinically. The absence of dextrose, which occurs in normal cerebro-spinal fluid, is characteristic of many cases of meningitis. In tuberculous meningitis a lymphocytosis is found, as also in tabes, in general paralysis, and in cerebro-spinal syphilis. Polymorphonuclear leucocytosis indicates an acute inflammatory condition of the meninges. It is possible, by means of lumbar puncture, to classify the various forms of meningitis, and to diagnose anomalous forms. The operation helps also to differentiate meningitis from cases of so-called meningism and from uræmia. In a few cases it has been possible to diagnose cerebral hæmorrhage from thrombosis or embolism. In cases of cerebral tumour a few cases are recorded where valuable information has been obtained. As a therapeutic agent lumbar puncture deserves more credit than it has yet received. It does temporary good in probably all cases of meningitis, and in some effects a permanent cure. It is a very valuable procedure in cases of increased intracranial pressure. Three cases may be cited—cerebral tumour, subacute nephritis, and fractured base of skull—in which it has led to recovery from coma, which would otherwise apparently have proved fatal.

TUBERCULOSIS (THE TUBERCLE BACILLUS).

Discovered by Koch in 1882, *Tubercle bacilli* occur as minute rods, often slightly curved, measuring 2·5 to 3·5 μ in length (sometimes long forms, 5 μ, are met with), and 0·3 μ broad—*i.e.*, very thin in proportion to their length—and they may show slight swelling at their ends. In young cultures the bacilli are usually homogeneous, no differentiation of structure being apparent; in old cultures they may appear either as long filaments, swollen, clubbed, branching (similar to streptothrix), beaded, or segmented. Is non-motile. Debatable whether spores

are formed or not, but bodies occur like arthrospores which may function as spores.

Staining.—The tubercle bacillus stains very slowly and faintly with ordinary stains; hence very strong *heated* solutions of aniline dyes are necessary, the best being the Ziehl-Neelsen method (see Chapter VIII., p. 137). When stained the bacilli are 'acid-fast,' a term which implies that they do not lose their colour for some time after staining on treatment by an acid decolorizing agent, such as 20 per cent. solution of sulphuric or nitric acid. This acid-fast property is due to a waxy substance in the bacilli, which is considered to make them very indigestible to phagocytes, thus accounting for their presence inside leucocytes for years, and it also protects the host from infection.

Other Acid-Fast Organisms.

1. The more important are the Leishman-Birt streptothrix and the actinomycosis streptothrix, as they sometimes occur in the sputum (see p. 69); the latter is only occasionally acid-fast.

2. Moeller's grass bacilli I. and II.—the former from Timothy-grass, and the latter from dust of hay-lofts.

3. Moeller's dung bacillus (mist bacillus); from manure. Distinguished by rapid yellow growth at 20° C.

4. Moeller's milk bacillus.

5. Petri's butter bacillus. Distinguished by its rapid growth on artificial media.

6. The smegma bacillus, readily decolorized by a minute's exposure to absolute alcohol, following sulphuric acid solution.

7. Cowie's bacilli in the secretions of the external genitals, mammæ, etc., of certain animals—cows, etc.

8. The bacillus of leprosy (Hansen's). Cannot be cultivated artificially.

Cultivation of the Tubercle Bacillus.—Is a facultative anaerobe, and will not grow in ordinary media. When first isolated requires serum or glycerinated serum

to grow on, and grows best at blood-temperature ; later it will grow on glycerinated media (agar, potato, broth), but very slowly, taking three weeks to show any growth at all. The growth on glycerine, agar or potato, is yellow, raised, warty, wrinkled, and dry, like lichen.

Resistance.—Considerable. Dried sputum may be infective for months. Bacilli, when completely dried, can resist a temperature of 100° C. for an hour, but if moist are killed at 70° C. in the same time, if this heat reaches them. In sterilizing milk, it may be noted that the scum or splashings on the top of the vessel may not reach this temperature, even although the milk may be boiling ; hence it is necessary to stir well to effect sterilization. Exposure to 5 per cent. carbolic acid kills in less than a minute. Direct sunlight is also said to kill rapidly ; and the dangers of spitting are more evident in the case of moist than dry sputum. Virulence is maintained for a long time, but if lost it can be readily restored by passage through a guinea-pig.

METHODS OF EXAMINATION.

1. MICROSCOPIC.—These are discussed under the respective secretions and excretions of the body. In the case of blood, see p. 3 ; sputum, see p. 69 ; milk, see p. 124.

In the case of urine or any other fluid, the liquid is let stand in a tall conical glass, the deposit pipetted off, centrifugalized, and film preparations stained by the Ziehl-Neelsen method. In urine the bacilli often occur in little clumps. The smegma bacillus should, however, be borne in mind, and to avoid it the urinary meatus should be cleaned and the first urine passed rejected.

2. INOCULATION.—This is the most suitable method, when, owing to a scanty supply of fluid, the microscope fails. Inoculation in a guinea-pig's thigh or peritoneum gives best results. Enlargement of the glands

occurs in ten days, and the animal usually dies within a month of acute tuberculosis. Bacilli are found in pure culture in the glands, liver, spleen, etc.

3. CULTIVATION.— Troublesome and impracticable. The best method to obtain pure cultures is to inoculate a guinea-pig and cut out a tubercular gland a month later; incise it and rub it over solidified blood-serum.

Distribution of the Bacilli in the Body and their Lesions.—The tubercle bacillus is the cause of phthisis, tubercular disease, and lupus. The latter can be experimentally produced by inoculation of the skin with infected saliva. In acute lesions the bacilli are numerous, especially at the spreading edge. In chronic lesions they are usually very few, and in pus from old-standing abscesses (psoas, etc.) the organisms are often extremely difficult to find. In local tuberculosis the tubercle nodules show bacilli extracellular in position, rarely in a radiate manner, or within the giant cells, lying near the margin. In acute miliary tuberculosis bacilli are often scanty, and occur towards the central parts of the nodule. In phthisis they are best demonstrated in the granulating walls of cavities, or lodged in the vicinity of thrombosed capillaries. In all tubercular lesions caseation depends on the action of the toxins, and not the non-vascularity of the tubercular nodules. Recent work on the tryptic ferments of the body, which, so to speak, aid digestion of the tissues, appears to show that the progress of caseation is dependent mainly on their abnormal increase in tuberculosis; and if this condition can be counteracted by the injection of some form of antitryptic body or vaccine, destruction of tissue can not only be lessened, but perhaps retarded.

The local lesion that occurs in a tubercular nodule is not necessarily characteristic of tubercular affections; it only shows the resistance of the tissues to an irritant, and a similar appearance is met with where the eggs of parasites are deposited. Again,

in tuberculosis the structure of the nodule varies according to the intensity of the action of the bacilli, the strength of dose, and their path of dissemination. If many bacilli are suddenly spread by the blood, a general disseminated miliary tuberculosis may be caused ; if a few get into the blood, the result is not so marked ; if many get into the lymph stream, a partial disseminated tuberculosis is the result ; if few, a local progressive process occurs. When the thoracic duct conveys infection, the result may be the same as when the blood-stream is involved. If many bacilli reach the lung from inspired air, lobar or lobular caseating pneumonia, generally of the lower lobe, occurs ; if few, the apex is generally infected first with a localized tuberculosis, and perhaps later by tubercular broncho-pneumonia, etc. Another form of spread is by leucocytes. This occurs when tubercle bacilli are phagocyted by polynuclear leucocytes, and are carried to some region where they settle down, form a colony, and become a source of irritation. Large mononuclears are attracted and surround the bacilli, and a giant cell results from the enlargement either of a single mononuclear, the nucleus of which undergoes proliferation without the protoplasm dividing, or, according to other observers, to the enlargement of a single or of fused epithelioid cells of the part, derived perhaps from the lining cells of capillaries. Lastly, the course of, or ultimate fate of, a tubercular nodule may be noted. If a favourable result is to occur, fibrous encapsulation takes place, perhaps aided by the encrustation of lime salts around the bacilli (calcification). In old calcareous nodules the bacilli may at times be demonstrated. In the unfavourable process the nodule undergoes degeneration, followed by necrosis and caseation. Local necrosis sets free the bacilli ; these are carried by phagocytes to neighbouring tissues, which become infected.

CHAPTER V

THE SEPTICÆMIAS—ENTERIC FEVER—MALTA FEVER—PLAGUE—INFLUENZA

THE septicæmias were formerly described as diseases produced by organisms which at some period of their existence were found in circulating blood; but recent work shows that bacteria may often be found in the blood as a result of *local* inflammation, as well as of a general infection.

ENTERIC OR TYPHOID FEVER.

MORPHOLOGY.—The *Bacillus typhosus* belongs to a large group of organisms which contain many members,—*i.e.*, *B. coli communis*, paratyphoid, Gaertner's bacillus, Shiga's bacillus, etc. It is a short bacillus, measuring 2 to 4 μ long and 0·5 μ broad, actively motile, with numerous flagella; can grow anaerobically; does not form spores; is Gram-negative; does not liquefy gelatine, clot milk, form gas in glucose media, or form indol. Involution forms are common in cultures (long threads with no segmentation, but of same diameter as the ordinary bacilli, and these may be flagellated). In old cultures motility may be lost, owing to the absence of flagella.

Growth.—In *gelatine stab* culture *B. typhosus* has a characteristic appearance. The growth spreads on the surface of the gelatine, and may reach the sides of the tube. It is of a bluish-white colour, and along the stab

there is an opaque whitish line of growth. There is no liquefaction and no gas production. On potato, at first the growth is invisible; later it becomes a pedicle, with a dull somewhat velvety surface, which may become brown if the potato is alkaline. In *broth* the growth is turbid and diffuse.

Conditions of Growth.—Best temperature is 37° C.; it flourishes at room temperature, but will not grow below 9° C. or above 42° C.

Vitality.—The thermal death-point is 53° C. for one hour. Like cholera, *B. typhosus* tends to die out in water, if a host of other organisms are present. A cesspool near Paris, known to contain typhoid bacilli, was closed for five months in winter, and the manure from it was then spread over a garden. Vegetables grown in this gave typhoid bacilli in pure culture from water that they were washed in.

In the body the *B. typhosus* has been known to form post-typhoid abscesses two years after enteric fever, and 'carriers' have been recorded as excreting the bacilli in one case forty years after typhoid, and in many cases for thirty years or less. Bacilli may also remain for years in the gall-bladder and urine; they may live for weeks in clothing, water, etc., and are very resistant in sand and dust. The bacillus can also remain as a saprophyte in the intestine, and need not necessarily cause disease in the 'carrier,' but on being excreted in fæces or urine it may infect healthy people.

Channels of Infection.—*B. typhosus* cannot cause enteric by inoculation, but infects either by ingestion or inhalation. Ingestion has more pathological evidence to prove it than inhalation, the chief evidence being the enlarged mesenteric glands and the intestinal lesions. People have also, either by accident, suicidal intent, or experimentally, swallowed laboratory cultures of typhoid, and have developed the disease. In ordinary cases of typhoid, the bacilli having passed the gastric juice—which, for some reason (lowered resistance), is at the time unable to kill

them off, or perhaps by reason of a large dose—they pass into the intestines. The lymphoid tissue of the Peyer's patches or solitary follicles becomes infected, and then they pass on to the mesenteric glands, and thence to the spleen, where they locate themselves in definite groups or colonies, giving rise to no local reaction* (grouping of leucocytes in the vicinity), which is peculiar, as all other infections of the spleen show local reactions. It may be noted in passing that splenic puncture for diagnostic purposes is not now done, as it is dangerous. The bacilli may also be found in the spots of the 'rose rash,' and in the gall-bladder, as well as in the circulating blood. They are difficult to find in the sputum of the secondary pneumonias that sometimes occur, but the sputum and *all* excreta—milk, sweat, urine, and fæces—of a typhoid patient are highly infective.

Elimination.—The bacilli are eliminated chiefly by the *fæces* in the *earlier* stages of the disease, and by the *urine* in the *later* stages. In from 20 to 25 per cent. of all cases a true typhoid bacilluria occurs.† It is not clear how the bacilli get into the urine, whether by the kidney or through the walls of the bladder. Urinary 'carriers' are not so frequently found as fæcal 'carriers,' and the carrying power of the former is said to be less lasting than in the case of the latter. In the post-typhoid abscesses of bone and affections of the testicle, etc., the typhoid bacilli act in symbiosis with either the staphylococcus, streptococcus, or *B. coli communis*.

Toxins.—These belong to the endotoxin group; the *B. typhosus* form no soluble toxin.

In Animals.—It is only recently that anything like clinical typhoid has been produced in animals by feeding. An-

* Why is there no local reaction in the spleen, whereas the lymphoid tissue of the intestine and mesentery is most seriously affected? Can any explanation be offered?

† *Vide* a report '*On the Spread of Enteric Fever by Urine and its Prophylaxis*,' by the present writer (*Journal R.A.M.C.*, vol. iii., pp. 1 to 16, 1904).

thropoid apes have been fed with a culture of the organism mixed in their food. *Post mortem* typical lesions were found in the intestines and glands of these animals.

Blood-Changes in Enteric Fever.

1. *Cells.*—Initial leucocytosis is found in the incubation period; it is absent during the same period in Malta fever. A fairly marked leucopænia is, however, found, 3,000 instead of 8,000 leucocytes per cubic centimetre being an average, with a relative increase of mononuclear or lymphoid cells, or both.

2. *Coagulability of the Blood.*—Our knowledge of this is due to Sir Almroth Wright, who made his investigations while Professor of Pathology at the Army Medical School at Netley. Two changes of vast clinical importance occur, which, if not checked, may lead to grave effects. Wright found that in the early stages of typhoid the coagulability of the blood is lessened; hence there is a tendency to *epistaxis*, and also bowel hæmorrhage. A pinhole perforation of an artery in the floor of an ulcer at this period may cause death. The lowered clotting power of the blood is due to a deficiency of its normal lime salts, and Wright recommends the administration of lime, either in the form of calcium carbonate or calcium lactate, the latter being the more palatable. Normally, clotting takes place in five minutes, but in the earlier stages of enteric fever it may be delayed to seven minutes. In the later stages, and during convalescence, the clotting power of the blood may be increased, and clotting may occur in three minutes or less. Post-typhoid thrombosis may be prevented by giving either citric acid or fresh lemonade, which precipitates the excess of lime salts in the blood, and makes it more fluid. Of several hundred cases of enteric fever treated by the writer, both the above recommendations were carried out in every case, the greatest possible success was achieved, and they can be confidently employed.

Agglutination.—Widal's agglutination method is of the greatest value for diagnosis of enteric fever. It was discredited for a time, owing to what were thought to be certain contradictory results, but these were really due to factors which were not recognized. The chief of these so-called fallacies are :

(*a*) *Insufficient Dilution.*—As normal human serum has often the power of agglutinating typhoid bacilli, if not sufficiently diluted with saline solution, it is necessary to work with dilutions of 1 in 50 and over.

(*b*) *Previous attacks of typhoid fever and persons inoculated with antityphoid vaccine* give agglutination reactions for years after the disease. Such people, coming to hospital with fever and giving the agglutination test, if erroneously diagnosed enteric fever, discredit inoculation and statistics.

(*c*) *The Strain of Culture used for Agglutination.*—Every strain of typhoid is not suitable ; some strains are auto-agglutinable—*i.e.*, clump with any serum. To guard against this always put up a control tube or control hanging-drop for purposes of comparison. It should also be remembered that the typhoid bacillus is only one species of a large group of bacilli that have a power of agglutinating each other—*e.g.*, *B. coli communis*, paratyphoid, Gaertner's bacillus, etc. Therefore it is not so necessary to estimate the fact of agglutination as the *degree of dilution* required to agglutinate : the higher the dilution, the more reliable the diagnosis for the bacillus concerned. If the paratyphoid bacillus be tested against *B. typhosus* serum, the reaction is slight ; but if either of these bacilli be tested against its own strain, they will agglutinate in high dilutions.

(*d*) *Day of Disease.*—It is also necessary to remember that the specific agglutination will not, as a general rule, occur in enteric fever *before* the seventh to tenth day of the disease ; therefore a negative diagnosis before this date does not mean that the patient has not got typhoid fever,

In some cases agglutination is delayed even until the third week.

(*e*) *Time is necessary for agglutination to occur.* The test may not come off at once, so it is not desirable to fix a standard for time. Allow one hour for the hanging-drop reaction, and twenty-four hours for the sedimentation test.

PROGNOSIS.—This may often be foretold by making daily agglutination experiments. An ordinary uncomplicated case of typhoid gives an agglutinating curve, beginning to rise at the end of the first week, or at the commencement of the second week, keeping at a fairly high level during the third week, and falling in the fourth week; from this period it remains at a considerable height for some time. In a severe case agglutination begins late (end of second week), does not rise high, and eventually falls off.

The agglutination curve of a person who has had a previous attack, or one who has been inoculated with anti-typhoid vaccine, remains at a certain height, and *stays stationary* from day to day and week to week. These cases may be erroneously diagnosed enteric fever, and discredit inoculation and statistics.

THE AGGLUTINATION TEST.—The method of making agglutination dilutions is as follows: Take a fine glass pipette and make a mark on it with a grease pencil, and proceed as follows:

1. First put up a control tube; this contains 1 volume of typhoid emulsion and 1 volume of normal salt solution. (N.B.—The control should contain *no serum.*)

2. Using the pipette, make a series of dilutions of the serum in normal salt solution:

1	vol. of serum	+ 4 vols.	salt	solution	= 1 in 5	dilution.
1	,, $\frac{1}{5}$,,	+ 1 vol.	,,	,,	= 1 in 10	,,
1	,, $\frac{1}{5}$,,	+ 4 vols.	,,	,,	= 1 in 25	,,
1	,, $\frac{1}{25}$,,	+ 1 vol.	,,	,,	= 1 in 50	,,
1	,, $\frac{1}{50}$,,	+ 1 ,,	,,	,,	= 1 in 100	,,
1	,, $\frac{1}{100}$,,	+ 1 ,,	,,	,,	= 1 in 1,000	,,

Other dilutions may be prepared thus :

1 vol.	of serum	+9 vols.	salt	solution	= 1 in 10	dilution.
1 ,,	$\frac{1}{10}$,,	+9 ,,	,,	,,	= 1 in 100	,,
1 ,,	$\frac{1}{100}$,,	+1 vol.	,,	,,	= 1 in 200	,,
1 ,,	$\frac{1}{100}$,,	+2 vols.	,,	,,	= 1 in 300	,,
1 ,,	$\frac{1}{100}$,,	+3 ,,	,,	,,	= 1 in 400	,,

Mix each dilution, and blow out into separate watch-glasses ; label these, and cover with other watch-glasses.

3. From the dilutions of serum fill sedimentation tubes by mixing in each case 1 volume of diluted serum with 1 volume of typhoid bacilli emulsion (this doubles the total dilution of each serum), allowing an unbroken column of the mixture to run up into clean pipettes, which are sealed. Allow to stand for twenty-four hours, and recognize agglutination.

Isolation of Typhoid Bacilli from the Blood.—This is a most important method of early diagnosis, as it is possible to obtain a definite diagnosis before it is possible to get Widal's reaction, or perhaps obtain the classical clinical signs and symptoms of the disease. With proper technique the bacilli can be isolated in every case, the chief factor being to take a sufficient quantity of blood (1 to 3 c.c.). Blood may be drawn from any vein (the median basilic is usually selected), and this must be placed in a large quantity of culture media (100 to 200 c.c. of broth) and incubated. Recently, using better media (ox-bile), incubating at blood-temperatures, and inoculating on Fawcus's media (*vide Journal R.A.M.C.*, February, 1909), the method has been much simplified. Blood-culture can also be used for forming a prognosis, as in ordinary uncomplicated cases the bacteria get fewer and fewer in the blood from day to day. In what is going to be a severe case they persist in large numbers, and are found in the fourth week still in the blood.

Antityphoid Vaccine.—The results of this are so satisfactory that its employment should be made compulsory for all Europeans proceeding abroad. In the *Journal*

R.A.M.C., February, 1909, Colonel Sir William Leishman, R.A.M.C., gives the following statistics of a number of British troops serving in epidemic areas who have been inoculated by the 'two-dose' method of recent years:

		Cases of enteric.	Deaths.
Inoculated	5,473 ...	21, or 0·4 per cent. ...	2
Non-inoculated (in same units)	6,610 ...	187, or 3 ,, ...	26

These figures speak for themselves: the inoculations reduced the incidence and deaths very considerably.

ESSENTIAL FACTOR OF INOCULATION.—This lies in the fact that the *two* doses of vaccine must be given; they are really part of one dose, ½ c.c. or 9 ♏ being given in the first injection, and 1 c.c. or 18 ♏ in the second, ten days later.

THE NEGATIVE PHASE AFTER INOCULATION.—As some capital has been made of this by the opponents of antityphoid inoculation, a few words here will not be out of place. Following inoculation a few days after injection, a period of a couple of days was said to occur wherein the person injected was thought to be more susceptible to typhoid before the subsequent immunity (positive phase) was established. This question has been closely investigated by experts, and there is absolutely no evidence to support it. It may possibly occur with increased doses of antityphoid vaccine, but has never occurred with the present vaccine or under the new system of dosage as employed in the British Army. It is therefore quite safe to inoculate, even in the face of an epidemic of enteric fever. During a raging epidemic of typhoid which occurred in the Maidstone Lunatic Asylum, Colonel Sir William Leishman, R.A.M.C., inoculated over a hundred of the attendants, and while none of those inoculated contracted the disease, many of those who refused inoculation not only contracted typhoid, but some of them died.

Treatment of Enteric Fever by Antityphoid Vaccine.—This has been carried out with good results at

home and abroad. Captain Smallman, R.A.M.C., contributes the results of a series of cases which he treated successfully in India in a paper published in the *Journal R.A.M.C.*, February, 1909. Colonel Simpson, R.A.M.C., has also met with success at Millbank Military Hospital, and Chantemesse, working in France, advocates the use of a special antityphoid *serum*, with which he has reduced the mortality of enteric to 4 per cent. He attributes its action to producing opsonification by giving it in extremely minute doses. Antityphoid serum acts on the same principle, and increases the leucocytic power against the bacilli. As antityphoid vaccine and antityphoid serum are quite different substances and cause different reactions on inoculation, particular caution is necessary to avoid confounding them when prescribing the dosage, as it varies very considerably for the two substances, and the results may be dangerously different.

MALTA OR MEDITERRANEAN FEVER.*

Malta fever is met with along the shores of the Mediterranean, Cuba, Philippines, Upper Egypt, South Africa, India, etc. In 1887 the specific micro-organism of the disease was discovered by Colonel Sir David Bruce, C.B., F.R.S., R.A.M.C., and from the spleen of patients who had died of the disease he cultivated the characteristic organism now known as the *Micrococcus melitensis*, and by means of inoculation experiments he established its relationship to Mediterranean fever. This discovery separated Malta fever from all the other continued fevers, and in 1896 it was embodied for the first time in the 'Nomenclature of Diseases' as a separate disorder. Owing to the great amount of inefficiency produced by the

* We desire here to acknowledge our indebtedness to Captain J. C. Kennedy, R.A.M.C., a member of the Malta Fever Commission, for several suggestions in this section.

disease amongst the naval and military garrisons at Malta, a Commission was appointed by the Admiralty, War Office, and the Civil Government of Malta in 1904, to carry out further investigations. This Commission discovered that the goats which supply practically all the fresh milk in the island suffered from the disease, and that infection to man was spread by drinking their unboiled milk. Arrangements were then made for boiling all milk, but this proved useless, as the 'Ortol' test, when applied, showed in many cases that the cooks could not be relied on. Orders were then issued in June, 1906, prohibiting the use of fresh milk, cream, butter, and cheese by the garrison, and preserved forms of these substances were taken into use in their stead. Results have shown that since these measures were adopted Malta fever has practically ceased to exist amongst the garrison, but amongst those of the civil population who still use goat's milk the disease is yet prevalent. This immense improvement in the health of the island, which is entirely due to modern scientific investigation, is perhaps one of the most brilliant examples of the value of bacteriology. Malta used to be shunned as a very undesirable station, and it has now become one of the healthiest of our foreign garrisons.

CHARACTERISTICS OF THE MICROCOCCUS MELITENSIS. —A small cocco-bacillus, slightly oval, usually occurring singly or in pairs, or in short chains in cultures, non-motile and non-sporing. It is about half the size of a staphylococcus, and is somewhat longer in one diameter than in the other.

Staining.—Stains with ordinary basic dyes, but is Gram-negative.

Cultivation.—Very slow-growing. On *agar* no evidence of growth is found until the third day, colonies being small and transparent in appearance. In *gelatine* the growth is also slow, taking two or three weeks, and no liquefaction occurs. The best medium for cultivation is

nutrose-litmus-lactose agar, or a mixture of agar and ascitic fluid.

Vitality.—Killed at a temperature of 60° C. for fifteen minutes. May live for a couple of months in dry fabrics or dust, seventy-two days in moisture, and in tap or salt water for a month.

Relationship to the Disease.—Always found in the spleen, where it occurs scattered, and not in clumps, as the typhoid bacillus is found. Also occurs in the glands (mesenteric, mediastinal, axillary, and femoral), and in the blood as early as the third day (like typhoid), and as late as the three hundredth day ; in the sputum when secondary pneumonia occurs, and in the urine, bile, bone-marrow, fluid of joints, pus from abscesses, etc.

Elimination.—By urine and also by fæces. The micrococci are easily found in most cases (Kennedy). The excreta are a probable source of infection in ambulatory cases.

SOURCES OF INFECTION.—Three possible channels.

1. *By Ingestion.*—Commonest method. Goat's milk and its products—butter, cream, cheese, etc. In Malta 40 per cent. of all goats were found infected and reacted to the serum test ; 10 per cent. were passing the coccus in either their milk or urine. There are no clinical symptoms of the disease in its early stage in goats, the infection being spread in their case by suckling. A case is also recorded where a child was infected *in utero*.

2. *By Inoculation.*—A possible but not common channel. In the case of goats a probable method of infection to man is from sores on teats to the milkman's hands (Kennedy). Inoculation to man has been proved by several instances of accidental inoculation in laboratories, one case being that of a R.A.M.C. officer who, when inoculating a horse, had the hypodermic needle driven into his hand by a movement of the horse's head, making a small puncture, which, notwithstanding cauterization, became infected, and he developed Malta fever. Monkeys

have also been inoculated with positive results. In an examination of mosquitoes, four out of 900 exhibited germs.

3. *By Inhalation.*—A possible but rare channel is by infected dust, but owing to the possibility of dust being ingested as well as inhaled, it is difficult to prove.

4. *By Contagion.*—Doubtful. Possible sexual congress?

BLOOD-CHANGES.—The following changes occur:

1. *Anæmia.*—Red cells diminished ($2\frac{1}{2}$ millions), hæmoglobin diminished, microcytes (occasionally) and normoblasts occur.

2. *Coagulability.* — Diminished, hence liability to hæmorrhages.

3. *Agglutination.*—Agglutinins present, and it is possible to get a Widal reaction. The rules as to technique applicable in the case of typhoid agglutination are also applicable to Malta fever, only much more rigid standards are required, and the observer should not be satisfied with a reaction in less than 1 in 50 dilutions, as Malta fever cocci may agglutinate with normal serum in dilutions less than this.* Controls must be carefully watched. The agglutination clinical curve gives better value as to prognosis than the typhoid curve, and a delayed or low agglutination in Malta fever should lead to a grave prognosis, not necessarily as regards a fatal issue, but it should be remembered that low agglutination is common in severe rheumatic cases (Kennedy).

DIAGNOSIS.—Take some freshly-drawn blood (2 to 3 c.c., or cocci may be missed) from the finger early in the disease, or some urine in later cases, and inoculate into nutrient broth, incubate, and examine.

TREATMENT.—Serum-therapy has not been satisfactory. Vaccine doubtful as yet. Treatment of *chronic*

* This, however, occurs only when the strain is open to doubt. A dilution of 1 to 10 to 1 to 20 is diagnostic, if the strain is reliable; 1 to 50 is too high in many chronic rheumatic cases (Kennedy).

cases by small doses of vaccine has been recently employed with success.

Prophylaxis.—A very great reduction—in fact almost a disappearance—of Malta fever has been brought about in the British garrisons at Malta and Gibraltar since the source of infection has been recognized. All milk is now boiled, and infected goats are being eliminated (p. 92).

ORIENTAL OR BUBONIC PLAGUE.

Plague is a septicæmia which can affect most mammals, but chiefly man and rodents (especially rats). The infective bacillus is transmitted by means of fleas, and perhaps through other channels.

Characters of the Bacillus.—Discovered during the Hong Kong epidemic by Kitasato and Yersin in 1894. A large cocco-bacillus, with rounded ends; staining more deeply at poles (bipolar staining); variable in size; larger in the body than in artificial culture; non-motile; non-sporing; aerobic, but can grow with difficulty under anaerobic conditions.

Growth.—Easily grown in ordinary media at 22° to 37° C.; does not liquefy *gelatine*. In *broth* tends to grow in chains, forming a strepto-bacillus as a flocculent deposit, and, if oil be added to the medium, the characteristic very fragile 'stalactite' growth is seen extending from the under surface of the oil globules. In *agar* it forms a white waxy, sticky growth, and involution forms occur in colonies containing bacilli—some of considerable length, others spherical, dumb-bell, and all varieties of shape. These involution forms are well seen if a 25 per cent. solution of common salt be added to the agar.

Staining.—Stain in all ordinary stains, but is Gram-negative.

Vitality.—Not very resistant; easily killed by heat or by drying (six days), and by exposure to the sun. Has remarkable resistance against cold. Dies in water in a few days.

Virulence.—Varies considerably; old laboratory cultures lose their virulence. There is a culture (three years old) in the Royal Army Medical College laboratory which, though quite innocuous, still retains its cultural reactions.

CHANNELS OF INFECTION.

1. *Inoculation.*—The disease can be produced by inoculation in man and animals. Accidental breakage of a tube in a Vienna laboratory caused several deaths; scratches and *post-mortem* accidents have also proved this, and that the period of incubation is about fourteen days (shortest two days, longest ten days). Pestis minor is a not uncommon affection amongst laboratory workers in the tropics. The bacilli occur in rats' blood and in their excreta, and spread from rat to rat by fleas (*Pulex cheopis*), and to man by fleas biting.

2. *Inhalation.*—This is especially prone to occur in pneumonic plague, which is very fatal, and it invariably reproduces the pneumonic type. Such cases must be carefully isolated and the sputum disinfected.

VARIETIES OF PLAGUE.

1. *Pestis Minor.*—This is a mild or abortive form of plague, seen at the beginning or end of epidemics; it occurs in 1 per cent. of cases, its chief characteristics being skin affections, malaise, slight fever, and enlarged glands.

2. *Bubonic Plague.*—Occurs in 90 per cent. of cases. The commonest glands to be affected, in order of occurrence, are (*a*) groin, (*b*) axilla, (*c*) submaxillary (in children).

3. *Cellulo-cutaneous* variety occurs in $3\frac{1}{2}$ per cent. of cases; it begins with a skin lesion, and buboes follow.

4. *Pneumonic or Pulmonary Plague.*—Occurs in 1 per cent. of cases; very fatal and extremely infectious.

5. *Septicæmic Plague.*—Occurs in 1 per cent. of cases; a severer form than bubonic; bacilli get into the blood-stream, instead of being stopped by the lymphatic glands. Septicæmic cases are always fatal.

6. *Intestinal Plague.*—Some observers describe a mesenteric gland infection caused by ingesting bacilli. Occurrence doubtful. Mesenteric glands enlarged.

Approximate Plague Mortality.

Pestis minor ...	1 per cent. of cases fatal.			
Bubonic	75	,,	,,	,,
Cellulo-cutaneous ...	63	,,	,,	,,
Pneumonic	99	,,	,,	,,
Septicæmic	99	,,	,,	,,

PATHOLOGICAL CHANGES.—The plague bacilli are carried to the nearest lymphatic glands and multiply. Buboes of large size occur, and form 'bags' of bacilli; when they suppurate staphylococci and streptococci are found with plague bacilli, but when they burst the pus becomes almost sterile, this being probably due to the action of the toxins, and is not so infective. If the case is proceeding to a fatal termination, the bacilli get into the blood-stream and a septicæmia is produced, so that hæmorrhage from the nose, stomach, etc., may be a means of spreading the infection. A plague rash may also occur of a hæmorrhagic or vesicular (blebs) nature; the blebs contain bacilli.

The bacilli appear in the blood in some cases before buboes form; in other cases they only appear in the blood during the late stages of the disease.

Post-mortem.—Most of the internal organs, especially the spleen, are found affected. The spleen is enlarged, black, and very fragile.

CHANGES IN THE BLOOD.

1. Polynuclear leucocytosis.
2. Decrease of coagulability and liability to hæmorrhages (rash and vesicles).
3. Presence of agglutinins. These are not of much clinical value, as thev only appear late in the disease.

SPREAD OF THE DISEASE.

1. *By Man.*—Rare, except by discharges, sputum, blood. From man to man it can be spread by fleas.

2. *By Animals.*—This is chiefly by means of the rat flea; rats also die from plague, and their bodies, fæces, urine, etc., may foul earth and the bacilli are not easily killed when dry. Rats eat dead rats. Rats are very susceptible to inoculation. Between epidemics it is possible that plague is kept going by transmission from rat to rat, and virulence is maintained by 'passage.' Fleas spread the disease from rat to rat, and from rats to man.

3. *By clothing*, merchandise and perhaps soil.

EPIDEMIOLOGY.—Plague is endemic in India, Arabia, Persia, China, Uganda and parts of Central Africa.

DIAGNOSIS IN DOUBTFUL CASES.

1. *Sputum.*—Look for streptococcic forms of bacilli.

2. *Blood.*—Put 5 to 10 c.c. in broth and incubate; scum falls to bottom (examine this), leaving broth clear.

3. *Blood-Films.*—Stain a thick film after dissolving out the hæmoglobin.

4. Inoculate a susceptible animal *—rat, mouse, or guinea-pig—and watch results.

5. Puncture an early bubo, stain, and examine a smear; or inoculate animals.

6. Examine for involution forms on salt agar, and for stalactites in broth.

PROTECTIVE INOCULATION. — Haffkine's vaccine method. Prophylactic vaccine is made by growing cultures in broth, with drops of oil on the surface; stalactites form, and the flasks are shaken every few days to break up these and induce fresh growths; incubate at temperature of 25° C. for about four to six weeks. Sterilize at 65° C. for an hour, and add 5 per cent. of carbolic acid. Contents well shaken to diffuse sediment, and put up for use in small bottles.

* In plague experiments great care must be taken to avoid being inoculated by fleas. The best pulicide is chloroform.

Dose.—1 to 2 c.c. of a month's culture. The Indian Plague Commission recommended a better method of standardization (according to amount of suspended matter), and improvements in ensuring freedom from contamination. Haffkine's results very good, but immunity only lasts for about six months.

Of 7,000 people inoculated, 0·01 per cent. got plague and 0·18 of these died.

Of 500 not inoculated at the same time, 6·7 per cent. got plague and 6·2 died.

It is quite safe to inoculate and give a moderate dose during an epidemic.

Anti-plague Curative Sera.—(1) Yersin's, (2) Lustig's. The Indian Plague Commission reported that a certain amount of advantage accrued from both of these. Yersin injected dead plague bacilli into a horse subcutaneously, then intravenously, and finally living plague bacilli. He bled the horse and injected its serum into man.

Lustig injected toxins into a horse in gradually increasing doses.

Strong has been trying a living vaccine; but living vaccines are dangerous, as they may contain tetanus organisms.

POST-MORTEM APPEARANCE OF INFECTED RATS.—Buboes present, greasy-looking mottled liver, and hæmorrhage on peritoneum.

INFLUENZA.

The influenza bacillus was discovered in 1892 by Pfeiffer, Kitasato, and Canon simultaneously; the first two observers found it in the bronchial sputum, while Canon observed it in the blood.

THE BACILLI OF INFLUENZA.—Occur in the sputum as very minute straight rods with rounded ends, 1·5 μ in length and 0·3 μ in thickness. They are non-motile, non-sporing, Gram-negative, and show no capsule.

Staining.—Stain feebly with basic aniline dyes, and are best stained by a weak solution (1 in 10) of carbol-fuchsin applied for five to ten minutes.

Cultivation.—Grow best on blood-agar at 37° C. Growth appears within twenty-four hours in the form of minute, almost transparent dots, like dewdrops.

Resistance.—Low. When dry will die off within forty-eight hours.

Dissemination.—Chiefly by fine particles of sputum, thrown out by an infected patient either coughing or sneezing.

MICROSCOPIC DIAGNOSIS.

1. Select a small portion of the greenish-yellow purulent matter in the sputum, and make film preparations ; stain by Ziehl-Neelsen carbol-fuchsin solution (1 in 10) for ten minutes.

2. Cultures. Blood-agar at 37° C. for twenty-four hours. No growth in ordinary agar at 37° C., or gelatine at 20° C. Generally difficult to grow and if successful, soon die. Strictly aerobic and powers of resistance very low.

3. Experimental inoculation in animals not conclusive, as animals are not liable to infection.

CHAPTER VI

THE INTOXICATION PROCESSES—DIPHTHERIA—TETANUS —CHOLERA

DIPHTHERIA.

THIS is an intoxication process, caused by the diphtheria bacillus, first described by Klebs and Löffler. The toxæmia is produced by toxins manufactured at the site of infection and these are absorbed.

CHARACTERS OF THE DIPHTHERIA BACILLUS.—A rather long rod-shaped bacillus, which shows on staining beading and clubbed ends. Involution forms are common ; a pear-shaped form is the commonest and is characteristic ; also long and short varieties, spherical, true branching forms (only met with in culture), showing its relation to the higher groups of bacilli, such as tubercle. Length is variable and depends on media and its reaction. Stain with granules (metachromatic) at each pole. Segmentation appearance. The bacilli in a film preparation lie every way, except end to end, often in parallel pairs, or forming **X**'s, due to division taking place laterally. Is non-motile and does not form spores. Is a facultative anaerobe.

Staining.—Gram-positive ; also stains well in methylene blue and Neisser's stain (metachromatic granules).

Growth.—Grows well anaerobically, best on serum, blood-agar, etc. Colonies small and discrete like dewdrops, semi-transparent, firm, grey, moist and shining. On *broth* and glucose media forms a scum or pellicle (delicate) and produces acid. (Hofmann's bacillus, which causes pseudo-diphtheria, does not do this to the same extent.)

Virulence.—Variable. In epidemics the bacillus may

occur in healthy people and infect others, and thus spread the disease. In an epidemic in the Duke of York's School 20 per cent. of the boys had diphtheria bacilli in their throats without being ill. Some observers think the bacilli cause no harm to a healthy unbroken mucous membrane.

RELATIONSHIP TO THE DISEASE.—In diphtheria, besides the specific organisms, staphylococci and streptococci are found in the membrane (symbiosis); local necrosis of the epithelium and inflammatory reaction occur; a large exudation of lymph, with fibrin in the deeper layers, coagulated around the superficial vessels, so that when the membrane is pulled off bleeding occurs. The bacilli are only found in the superficial layers of the membrane as a rule, and streptococci (when present, prognosis bad) and staphylococci deeper.

THE TOXINS CAUSE—1. Hyaline changes in the blood-vessels, giving rise to hæmorrhage and œdema.

2. Changes in the epithelium of the uriniferous tubules of the kidneys, giving rise to albuminuria.

3. Action on cardiac muscle, which may lead to heart failure.

4. Affinity for central nervous system, causing local paralysis.

The filtrate from a pure diphtheria culture, if inoculated, causes the same results as the bacilli, thus proving that the poisons are in the toxins; or again, if a dose of toxin be neutralized by antitoxin, a neutral combination is made, and if this mixture is inoculated, there is no result.

VITALITY.—Diphtheria bacilli stand drying very well, but are easily killed by heat.

SOURCES OF INFECTION.—Inhalation, causing local growth in the throat. Wounds may become infected by inoculation.

DIAGNOSIS.

1. With a sterile swab (free from antiseptics) take some of the mucus from the affected part, rubbing behind the

tonsil and soft palate. No antiseptic spray or gargle should be used by the patient for some time (three to four hours) before the swab is taken.

2. Examine a smear microscopically, staining with Gram, Neisser's stain, or methylene blue.

3. Make culture on blood-serum, or coagulated antitoxin serum, or white of egg. Incubate for twenty-four hours (diphtheria outgrows all other organisms) and the characteristic colonies appear, which are confirmed by staining and the fermentation of dextrine.

4. Inoculate a guinea-pig with 1 or 2 c.c. from a broth culture and it dies in twenty-four hours. *Post-mortem signs :* Local œdema; peritoneal, pleural and pericardial effusion ; congestion of suprarenals, lungs, and spleen.

PSEUDO-DIPHTHERIA.—The following bacilli resemble the diphtheria bacillus : Hofmann's bacillus (shorter, not beaded, and does not stain with Neisser's stain), xerosis bacillus (found in healthy conjunctiva and follicular conjunctivitis), and other diphtheroids (chromogenic bacteria).

DIFFERENCES OF B. DIPHTHERIÆ AND ITS PSEUDO-FORMS TABULATED.

B. Diphtheriæ.	Hofmann's B. Pseudo-diphtheriæ.	B. Xerosis.
1. Generally long	Short	—
2. Forms acid in broth glucose in twenty-four hours	No acid	No
3. Granules by Neisser	No	No
4. Gram-positive	Yes	Yes
5. Kills guinea-pigs	No	No
6. Involution forms	Not common	—
7. Anaerobic	No	—
8. Ferments dextrine	No	No
9. Segmentation	Not so marked	—

PREPARATION OF ANTITOXIN.—Inoculate a young horse, free from glanders and tubercle, using small doses of a dead culture; increase injections and dose; test serum in six months' time, draw off 5 to 6 litres of blood, and standardize serum into units (1 unit will neutralize 100 minimum lethal doses: a minimum lethal dose is the smallest amount of toxin that will kill a 250 gramme weight guinea pig in four days).

PROPHYLAXIS.—As diphtheria toxins act rapidly in the human subject, and form a very close and binding chemical combination in the tissues, diphtheria antitoxin must be injected without delay. If, however, time has been lost, this chemical combination may be broken up at times by a *large* dose of the antitoxin. A dose of 600 to 1,000 units of antitoxin used to be considered ample and lasts for three weeks; 10,000 units are sometimes given now, but the usual dose is about 4,000 units. In old and late cases it should be given intravenously, as it can better unlock the toxin combination formed in the body; but ascertain if carbolic acid or other preservatives are present in the antitoxin; if present, do not give it intravenously, or coagulation of the blood will occur, with disastrous results.

NEISSER'S STAIN.—This is a good stain for diagnostic purposes. Stain for five seconds in a mixture of 2 parts 'A' solution to 1 part 'B' solution.

'A' Solution.

	Parts.
Meth. blue (powder)	1
Absolute alcohol ...	50
Glacial acetic acid	50
Aqua destill. ...	1,000

'B' Solution.

	Parts.
Crystal violet ...	1
Absolute alcohol ...	10
Aqua destill. ...	300

Wash at once in water.

Counterstain for about three seconds in cresoidin (1 part of cresoidin dissolved in 300 parts of warm water and filtered). This is a good stain for swabs from throats and young cultures; it is not satisfactory for old cultures.

TETANUS.

Tetanus, or lockjaw, is a specific, infectious, very fatal disease, occurring naturally chiefly amongst horses and

men, but other animals may become affected. In the body the *bacilli* are only found at the site of infection, which is usually a wound, often a mere scratch or the bite of a tropical insect. The wound is infected by earth or dung, which is the habitat of the bacilli of tetanus in nature. The disease kills by its *toxins* circulating in the blood-stream, and hence the changes are those of an intoxication rather than those of a septicæmic process. Whatever the nature of the toxins is, they are undoubtedly the most powerful poisons known, as it has been calculated that $\frac{1}{2000}$ of a grain will kill an adult man. Ehrlich has shown that, as well as the predominant spasm-producing toxin (tetano-spasmin), there is also a hæmolytic or crude toxin, capable of breaking down red blood-corpuscles (tetano-lysin). A significant feature of the action of tetanus toxin is the occurrence of a definite incubation period between the inoculation and the appearance of the first symptoms. From four to fourteen days after injection the first symptom to occur is the gradual onset of general stiffness and tonic spasms of the voluntary muscles, commencing in those of the jaw and back of the neck and extending later to all the muscles of the back. The disease is generally fatal, the tonic spasms succeeding each other at rapid intervals as the condition gets worse. *Post-mortem*, little is found save congested areas in the medulla and spinal cord.

BACILLUS TETANI.—In the body the bacilli are only found at the site of infection; in the discharge or necrotic tissue of the wound microscopic diagnosis can be made from film preparations from these, provided the characteristic spore ('drum-stick') formation is present. The spores are round, and form as a knob at one end of each bacillus, giving them the appearance of a drum-stick. In films stained with a watery solution of gentian violet or methylene blue the spores stain at their periphery in ring-like forms; if films be heated, spores become separated from their bacilli. If the characteristic 'drum-sticks' are absent—*i.e.*, if sporulation is absent—the bacilli appear as

rods, and are not recognizable from other organisms, many of which are always present in a tetanus wound.

Staining is effected by any of the ordinary stains, and also by Gram's method. The bacilli are 4 to 5 μ long and 0·4 μ thick, and are slightly *motile*, possessing numerous delicate flagella, attached both at the ends and sides; these can only be demonstrated by special staining methods.

Isolation is somewhat troublesome, and to obtain pure culture advantage is taken of the resistance of the spores to heat. Pus is inoculated on a sloped tube of inspissated serum or a deep tube of glucose agar, and incubated at 37° C. for forty-eight hours; this allows of spores forming. The culture is now heated to 80° C. for an hour to kill all organisms except the tetanus spores. Inoculation is made from these on glucose gelatine, and roll cultures made and incubated in an atmosphere of hydrogen (the bacilli being anaerobic) at 22° C. for four to five days; and if other obligatory and facultative anaerobes which may be present have not grown faster and overgrown the tetanus bacilli, the method may give pure cultures. The latter, when subcultured in deep upright *glucose gelatine*, shows a growth commencing half an inch below the surface, consisting of fine straight threads, radiating from the central needle track, with slow liquefaction of the gelatine and slight gas formation. In *agar* the growth shows small nodules along the needle track, with lateral offshoots, slight gas formation, but no liquefaction. The conditions of growth are strictly anaerobic, optimum temperature 37° C. and minimum 14° C. Spores are extremely resistant; they can withstand boiling for five minutes, and can remain virulent in the dry state for many months and have also great resistance to antiseptics.

*Microscopic Diagnosis.**—The routine bacteriological

* Tetanus is diagnosed by its clinical signs, and the microscopic diagnosis of the organisms has its chief value in showing where infection has its site, and consequently the part requiring surgical treatment.

technique to be carried out in cases suspected of tetanus should be as follows :

1. Note if the wound has been soiled by earth or dung.

2. *Microscopic examination* of the pus or necrotic tissues of the wound. Stain with carbol-fuchsin diluted with 5 parts of water. Look for 'drum-sticks.' Care must be taken to distinguish these from other thicker bacilli with oval spores placed at a short distance from their extremities, as these are common in earth, etc., and also occur as putrefactive anaerobes in septic wounds.

3. *Cultivation.*—If microscopic examination of the pus, etc., from the wound fails, a good plan sometimes is to inoculate some of this material in a deep tube of glucose medium and incubate for forty-eight hours, and examine microscopically for 'drum-sticks.'

4. *Inoculation.*—Take some of the pus and inoculate *subcutaneously* either guinea-pigs or mice. In cases of post-operative tetanus attention is also invited to the examination of cerebro-spinal fluid, and also the examination of catgut ligatures to see if organisms can be isolated.

Antitetanic Serum.—This should be employed as early as possible. The antitetanic serum made in the Pasteur Institute has a strength of 1 in 1,000,000,000, and 50 to 100 c.c. is injected in one or two doses.

CHOLERA.

Cholera is an intoxication process of a similar nature to that produced in diphtheria ; it is caused by the 'comma' bacillus, or *Vibrio choleræ asiaticæ*, discovered by Koch in 1883, and published in his researches in 1884. The bacilli are only found in the intestine, except in rare cases of long duration, when they may enter the blood-stream. They form powerful toxins, causing a general poisoning (toxæmia), in which the circulatory and thermal regulating mechanisms of the body are especially affected.

Characters of the Cholera Bacillus.

The vibrio is a thin, short, curved rod, variable in length (average size 1·5 to 2 μ long and 0·5 μ broad), curved in one

direction. Spiral and **S**-shaped forms may also be seen (spirillum). Involution* forms appear in most forty-eight hour cultures (spherical, clubbed, etc.), so that they may appear to be contaminated, but months afterwards colonies of the pure type will be got from these on agar. The bacillus is actively motile in fresh cultures from cholera stools, motility being due to a single terminal flagellum. Some observers have described flagella at each pole or others from the sides, but these occur in strains which are not true cholera.

Growth.—Grows rapidly on all media. Peptone 2 per cent. and salt 1 per cent. in water is the best medium. In this it grows rapidly, producing indol in eight to twelve hours (*B. coli* takes four days). *Gelatine* plates give characteristic colonies in twenty-four to forty-eight hours; these have an irregularly granular or furrowed outline, and as they grow larger their surfaces have been compared to fragments of broken glass; later (four to five days) liquefaction occurs, and the colony sinks into the cup, which has a clear-cut, sharp margin. In *gelatine stab* a bell-shaped area of liquefaction occurs, and later liquefaction is general. In milk,† it causes no clotting for several days, then acidity. The most important test for cholera is the *cholera red reaction*. If cholera is grown in peptone broth it produces indol and nitrites, and if a few drops of *pure* nitric or sulphuric acid are added, a reddish-pink colour is produced.

Staining.—Stains readily. Löffler's methylene blue or weak carbol-fuchsin is especially suitable. Löffler's blue is made from a saturated solution (alcoholic) of methylene blue, 30 c.c., caustic potash in diluted water (1 in 10,000), 100 c.c. The cholera spirillum is Gram-negative.

* Involution forms (especially cocci) are more common in cholera than in any other organism.

† The organisms which spread disease by milk do not cause clotting of milk—viz., cholera, diphtheria, typhoid, dysentery, and tubercle.

Vitality.—Low. It is easily killed at 60° C. for ten minutes, and also easily killed by ordinary disinfectants and weak acid solutions. The latter is of importance, as it should be possible to apply it to water in epidemics. Its life in water is variable : in well-water it lives three to eight days, in sterile water possibly for a year. If other germs are present, it only lives a short time (seven days). It has little resistance in the dry state ; it is not carried by dust, but by water.

Relationship to the Disease.—The bacilli are found in the rice-water stools of nearly every case.* They are very sensitive to the acid of the gastric juice, which kills them, unless it is deficient or a large dose of cholera germs is taken. In the intestine conditions for growth are favourable, and it first acts as a saprophyte and then enters the tissues, limiting itself to the superficial layers of the mucous membrane; in rare cases it reaches the internal organs—liver, gall-bladder, etc. In the intestine a moderate inflammatory reaction sets up desquamation of epithelium ; flakes of lymph are thrown off, and passed in the rice-water stools.

Toxins.—The constitutional symptoms are due to the absorption of toxins, which are intracellular or endotoxins. They abstract water from the tissues, causing cramps, etc.

Experiments in Animals and Man.—Not satisfactory in animals. Koch fed rabbits on the bacillus in food, first neutralizing their gastric juice, and then slowed peristalsis of intestine with opium or morphia ; large doses had to be given to produce rice-water stools and the disease. In man, suicides have produced the disease by swallowing cultures. Inoculation will not produce it ; Haffkine uses living cultures for vaccines.

Paths of Infection.—Inhalation unlikely, as the vibrios die quickly when in the dry state. Ingestion, by water or contaminated food, also cholera excreta, and possibly flies, are the chief channels of infection.

* In some malignant cases occurring in severe epidemics the bacilli may be absent from the stools.

Blood-Changes.—If the patient survives, agglutinins* are formed in the blood, but not early enough for diagnosis; they may be useful for diagnosis in doubtful cases.

Bacteriological Diagnosis.—Select a lymph floccule from the rice-water stools, and examine it direct or by culture.

Direct Examination.

1. Look for organisms in a hanging-drop.

2. Make smears and stain; the vibrios are decolorized by Gram. By ordinary stains the vibrios will be seen arranged in the characteristic 'fish-in-stream' appearance (heads all the same way). This is only seen in fresh specimens, and not in culture strains.

Culture Examination.

1. Prepare a peptone salt solution, and inoculate a floccule into it. Incubate at blood temperature. If cholera is present, in twelve hours a distinct turbidity occurs; the cholera germs will be found on the uppermost layers of the fluid, if other germs are present.

2. Apply the 'cholera red' test. This is not absolutely diagnostic, but is good enough for practical purposes.

3. Inoculate gelatine plates, and in four to five days liquefaction occurs.

4. Inoculate milk and other media.

5. *Pfeiffer's Phenomenon.*—A loopful of the bacillus to be tested is taken from a recent culture on agar. It is added to dilute anticholera serum, and the mixture is injected into the peritoneal cavity of a young guinea-pig weighing 200 grammes. At the end of twenty minutes or half an hour a sample of the peritoneal fluid is withdrawn, and a hanging-drop of it is examined. If the bacilli injected are cholera, the anticholera serum, acting in a favourable medium (peritoneal cavity of guinea pig), has produced *bacteriolysis*, which is effected by the bacilli first becoming motionless, then agglutination and swelling up into spherical globules, and ultimately breaking down and disappearing. If the germs are not cholera vibrios, the

* Late agglutination also occurs in plague.

cholera vaccine has no effect on them, and on removal from the peritoneal cavity they are found motile. Control experiments should in all cases be made by injecting similar quantities of the bacilli with an equal quantity of saline solution (instead of cholera serum) into another guinea-pig's peritoneal cavity; there should be no alteration of the organism on withdrawal.

Serum-therapy.—Haffkine was the first to bring out a vaccine, and it gives extremely good protection in one to two days after inoculation, reducing incidence to disease, but not mortality, having no dangers, and causing immunity to last well over a year. Haffkine first prepared a weak vaccine (a living vaccine, and the only living vaccine used) by passing a current of sterile moist air over the surface of cultures as they grow on agar; a dose of this is given, which produces only a local reaction, and five or six days later the strong vaccine is given, the virulence of which has been increased by passage through twenty to thirty guinea-pigs (by peritoneal injection, incubating for ten hours, and injecting another guinea-pig, and so on), and then grown on the surface of agar, emulsified, standardized and an antiseptic added. The dose was about 2 or 3 c.c. of the weak vaccine, followed in four or five days by the strong vaccine. Now only the strong vaccine is used.

Other Spirilla resembling Cholera.

I. *Metchnikoff's Vibrio.*—Obtained from epidemic diarrhœa in fowls at Odessa (not chicken cholera). This has the same shape and staining, but differs from it in—

1. It can grow in absence of oxygen (cholera can only do this with difficulty).
2. Liquefies gelatine more rapidly.
3. Coagulates milk, and grows more readily.
4. Inoculation in animals or pigeons causes septicæmia; true cholera does not.
5. Pfeiffer's test gives the negative reaction.

II. *Finkler-Prior's Spirillum.*—This is found in the stools of cholera nostras, but there is no evidence that it

has any causal relationship to this or to any other disease. The vibrio closely resembles Koch's spirillum, but is thicker and more pointed at the ends. It differs from true cholera in that—

1. Grows more quickly on gelatine, liquefies it in twenty-four hours, and the liquefaction spreads more rapidly.
2. Cholera red reaction is not obtained until very late.
3. It causes a fœtid odour on all media.
4. Coagulates milk.
5. Pfeiffer's reaction negative.
6. On intraperitoneal injection it is less virulent than cholera.

Differences between Cholera and the Spirilla of Metchnikoff and Finkler-Prior tabulated.

Cholera.	Metchnikoff.	Finkler-Prior.
Liquefies gelatine slowly (four to five days)	More rapidly	Much more rapidly (twenty-four hours)
Does not clot milk	Clots milk	Clots milk
Pathogenic to man, but not to fowls	Pathogenic only to fowls and pigeons	Pathogenic to fowls
Pfeiffer's reaction positive	Negative	Negative
Forms indol and nitrites	Forms indol slowly; does not form nitrites	Forms indol later still; does not form nitrites
Air-bubble in stab gelatine	—	No air-bubble

There are also considerable numbers of intermediate forms between the above spirilla, which closely resemble cholera vibrios.

CHAPTER VII

VOMIT — FÆCES — URINE — MILK—VAGINAL AND FALLOPIAN SECRETIONS: THEIR PATHOLOGICAL BACTERIA AND PARASITES — BACILLUS COLI COMMUNIS —ANKYLOSTOMIASIS—TRICHINOSIS

THE VOMIT.

THIS may occasionally show non-pathogenic mould fungi, scattered gonidia, fission fungi (bacilli and micrococci) of every description. Of the pathogenic vegetable parasites, the thrush fungus (sometimes), diphtheria (rarely), and plague bacilli, when a gastric hæmorrhage occurs, may all in turn be found in the vomit. Of the animal parasites, *Ascaris lumbricoides*, *Oxyuris vermicularis*, *Ankylostoma duodenale*, trichinæ (rare), hooklets of echinococcus, hydatid cysts and dipteral larvæ, occasionally may be found.

THE FÆCES.

There is no excretion of the body so liable to be infested with parasites as the fæces. Some are animal, others vegetable ; many of the latter are derived from the intestinal fluids, in which some aid in the processes of digestion. The bacteria of the small intestine act on the carbohydrates, producing ethyl alcohol, while those of the large intestine aid in the final breaking up of proteids.

The parasites generally may be classified as follows:

A. Vegetable parasites.

B. Animal parasites.

(A) VEGETABLE PARASITES OCCURRING IN THE FÆCES.

These may be broadly divided into pathogenic and non-pathogenic ; but some of the latter may on occasion

become pathogenic — for example, the *Bacillus coli communis*.

NON-PATHOGENIC VEGETABLE PARASITES.

1. *Moulds.*—The thrush fungus occasionally occurs in children suffering from thrush, but is of little clinical importance.

2. *Yeasts* (*Saccharomycetes*).—Round or oval, lying in groups of threes or fours, and often show budding forms.

3. *Bacteria* (*Fission Fungi*).—Occur in millions; micrococci predominate in solid fæces, bacilli (many motile) in fluid stools. The *Bacillus coli communis* most important (see p. 125). *B. subtilis* also occurs. The *Clostridium butyricum* of Nothnagel, which resembles yeast fungi composed of large round cells, often like a string of beads, are important, as they stain with the Ziehl-Neelsen fluid (acid-fast).

PATHOGENIC VEGETABLE PARASITES.

The fission fungi (bacteria) that may occur in the fæces are:

1. *Cholera vibrio* (Koch), see pp. 107 to 112.
2. *Bacillus typhosus* (Eberth), see pp. 83 to 91.
3. *Bacillus tuberculosis* (Koch first demonstrated the presence of this organism in the fæces in a case of tubercular ulceration of the intestine); see also pp. 78 to 82.
4. *Bacillus pestis*, see pp. 95 to 99.
5. *Bacillus dysenteriæ* (Shiga).

(B) ANIMAL PARASITES OCCURRING IN THE FÆCES.

Leuckart* classifies these as follows:

A. PROTOZOA.

I. RHIZOPODA.

(*a*) *Monadines.*

(*b*) *Amœba coli.*

* R. Leuckart, 'Animal Parasites,' vol. i., part i., second edition, p. 221 (1879-86).

II. SPOROZOA.

III. INFUSORIA.

(*a*) *Cercomonas intestinalis.*

(*b*) *Trichomonas intestinalis.*

(*c*) *Paramæcium coli.*

B. VERMES.

I. PLATODA.

(*a*) CESTODA (TAPE-WORMS).—*Tænia solium T. saginata* (*mediocanellata*), *T. nana*, *T. diminuta*, *T. cucumerina*, *Bothriocephalus latus.*

(*b*) TREMATODA (FLUKE-WORMS).—*Distomum hepaticum*, *D. lanceolatum*, *D. sinense*, *D .felineum.*

II. ANNELIDA.

(*a*) FAMILY ASCARIDÆ.—*Nematoda* (*round-worm*) : *Ascaris lumbricoides* (*common round-worm*) ; *A. mystax* (*cat*), *Oxyuris vermicularis* (*common thread-worm*).

(*b*) FAMILY STRONGYLIDÆ. — *Ankylostomum duodenale* (*Egyptian chlorosis*).

(*c*) FAMILY TRICHOTRACHELIDÆ.— *Trichocephalus dispar* (*whip-worm*), *Trichina spiralis.*

(*d*) FAMILY RHABDONEMA (ANGUILLULA) STRONGYLOIDES. — *Anguillula intestinalis.*

C. INSECT LARVÆ.

Piophilvea casei (*cheese maggot*).

Drosophilia melanogastra (*curdled milk pupa*), *etc.*

MONADINES.—These are pear-shaped protozoa with a long, actively motile terminal process. When dead they

appear circular. They occasionally occur in the stools in phthisis, enteric fever, and heart disease. Their pathological significance is unknown.

AMŒBA COLI.—The causal agent of one form of hepatic abscess, enteritis, and dysentery. They appear as contractile protoplasmic bodies with amœboid movement, and are best obtained from the smaller pellets of mucus in the stools. No distinct cell membrane, internally hyaline in parts, coarsely granular in others; have a large round nucleus, and show hyaline vacuoles. Diameter varies from 10 to 40 μ. Microscopic examination should be made of fresh stools, as the amœbæ disintegrate rapidly, and care should be taken not to press the cover-glass on the slide, and to keep the slide warm, which aids amœbic movements. Films are best stained by Leishman's fluid.

COCCIDIUM PERFORANS.—Elliptical, 0·022 mm. long. Capsule thin; granular nuclei lying in groups internally; found burrowing the intestinal mucous membrane.

CERCOMONAS INTESTINALIS.—Pyriform; has eight flagella of variable length; nucleus distinct. Found in diarrhœic conditions, also in cholera and enteric fever. Encysted forms also described.

TRICHOMONAS INTESTINALIS.—Pear-shaped; larger than *C. intestinalis;* has ciliated disc at one end.

PARAMÆCIUM COLI (BALANTIDIUM COLI).—Oval; 45 μ by 31 μ; covered with cilia; has nucleus and two contractile vesicles. Causes diarrhœa and ulceration of colon. Stools must be examined very fresh, otherwise disintegration occurs. Described by Major C. Lane, I.M.S., as occurring amongst gaol prisoners in India.

(*a*) **Cestoda**.—The presence of tape-worms is confirmed by finding either the head or proglottides in the fæces; the former is rare, unless an anthelminthic is given. The head or proglottides are best examined under a low-power microscope after mounting in glycerine. In cases where proglottides cannot be found, ova may be

demonstrated in the stools. A small quantity of the latter is taken and put into a test-tube three-quarters full of normal salt solution, shaken well up, and then let stand for half an hour. The sediment is then collected and washed several times with fresh saline, and some of this examined. Cestodes have two distinct stages of existence, in which the adult form is generally found in the intestines of one species of animal, the host, and the embryo usually occurs in the intestines of another species, the intermediate host. The head of the adult worms have *four suckers*, by which they attach themselves to the mucous membrane. In front of the suckers is a *proboscis*, or rostellum, surrounded by two rows of hooklets. The head passes into a narrow neck, and the body is made up of a number of flattened segments, or proglottides. The body contains no alimentary canal, but at each side are two longitudinal tubes, which represent the *water vascular* system. Each proglottis is bisexual and contains the respective sexual ducts—testes, vas deferens, penis, uterus, ovaries, oviducts, and vagina. The distal proglottides, containing the fertilized ova, break off and are passed in the fæces. The proglottides then disintegrate and the embryos, each provided with six hooks, are swallowed by the intermediate host. The digestive juices dissolve their containing capsule, and the embryos bore their way through the walls of the intestine, and settle in the viscera and muscles, where the hooklets disappear, and a cystic stage follows (*Cysticercus cellulosæ*, or bladder-worm). In this stage an immature head (scolex) is seen, which is invaginated into a cavity containing a clear fluid. The cyst (or cysts) is surrounded by a fibrous capsule. Should the flesh containing this cystic stage be eaten uncooked, the cysticercus develops into a mature worm, its head becoming everted, the cyst disappearing, and four suckers developing, which fasten on to the mucous membrane; finally, proglottides develop, and the worm reaches its adult form.

HYDATID CYST.—This is the intermediate stage of *Tænia echinococcus* (a tape-worm found in the dog) forming the well-known *hydatid cyst* in man. The encapsuled embryos in this case have six hooklets on the head. The gastric juice of the person swallowing them dissolves the capsule, and the worms are set free. They bore their way into the tributaries of the portal system, and come to rest in the liver or elsewhere. The hooklets are now cast off, and the worm enters a cystic stage. The walls of the cyst are formed of concentrically laminated layers (diagnostic), termed the *germinal membrane*. The fluid in the cysts contains the loose hooklets (diagnostic), and smaller cysts (*brood capsules*), containing immature heads (*scolices*), crowned with hooklets, which are analogous to the *Cysticercus cellulosæ* of other tape-worms. The entire hydatid tumour is surrounded by dense fibrous tissue.

Ova of Tape-worms.

Tænia solium.—Oval; 0·03 mm. in diameter, 0·036 mm. ong; thick capsule, radially striated. When mature, embryos inside may be seen to contain hooklets.

Tænia mediocanellata.—Resemble ova of *T. solium*, but are more elongated, and show a primordial yolk membrane.

Tænia nana.—Size, 0·03 to 0·04 in diameter. No striation in shell, but it consists of a double membrane, within which the embryo lies, provided with five to six hooklets.

Tænia cucumerina (*elliptica*).—Diameter 0·05 mm. Contain embryos with hooklets.

Bothriocephalus latus.—Oval, measure 0·07 by 0·045 mm. Brown capsule, with a lid at one end. Ova found free in fæces.

Cysts of echinococcus and hooklets may be found in the fæces when a hydatid cyst has burst into the intestine.

(*b*) **Trematoda.**—The following fluke-worms (see p. 120) have been found, although very rarely, in the intestines or biliary passages of man, and may pass into the fæces.

APPROXIMATE MEASUREMENTS, ETC., OF THE MORE IMPORTANT TAPE-WORMS TABULATED.

Name.	Intermediate Host.	Length.	Number of Proglottides.	Rostellum.	Hooklets.	Suckers.	Size of Head.
T. solium	Pig	About 10 feet	800	Present	26	4	$\frac{1}{40}$ inch.
T. medio-canellata	Ox	15 feet	1,000	Absent	Absent	4	$\frac{1}{12}$ inch.
T. echino-coccus ...	Man Sheep	$\frac{1}{4}$ inch	3	Present	Present	4	$\frac{1}{30}$ inch.
Bothrio-cephalus latus ...	Sturgeon Pike Trout	20 feet	3,500	Absent	Absent	2	$\frac{1}{10}$ inch.
T. nana ...	Insects Myriapoda	2·5 to 10 mm.	150	Present	22 to 30	4	0·3 mm.
T. cucume-rina	Dog-louse	15 to 50 mm.	Over 40	Present	Numerous, in 5 to 6 rows	4	—

1. *Distomum hepaticum.*—Leaf-shaped; measures 30 by 12 mm. One sucker on head and another ventral. Prickly scales on back (diagnostic). Ova, oval; size 0·13 by 0·08 mm. with flat lid, like *Bothriocephalus*, but shell browner in colour.

2. *Distomum lanceolatum.*—Lance-shaped; size, 8 to 10 mm. long and 2 to 0 3·5 mm. broad. Ova 0·04 mm. long, by 0·03 mm. broad.

3. *Distomum rathonisi.*—Resembles *D. lanceolatum*, but is larger (25 mm.).

4. *Distomum sinense.*—Size, 18 mm long. Ova, oval; 0 03 long, by 0·016 mm. broad; has a lid and a spike at opposite end. Endemic in Japan; causes severe hepatitis.

5. *Distomum felineum.*—Resembles *D. sinense.* Ova have lid at the pointed end. Usual hosts—man, cat, and dog.

II. ANNELIDA.

(A) Family Ascaridæ.

Nematoda.—The following round-worms and their ova occur in man.

1. *Ascaris lumbricoides* (Common Round-worm).—This is a tapering cylindrical worm, the male measuring 250 mm. and the female 400 mm. long. The head has three conical lips, with minute teeth. Tail of male bent like a hook and has papillæ. Infests small intestine. Also found in cattle and sheep.

2. *Ascaris mystax.*—Infection derived from cats. Resembles *A. lumbricoides*, but is smaller. Pointed head. Two lateral wing shaped processes at head end. Size: male, 45 to 60 mm. long; female, 110 to 112 mm.

Oxyuris vermicularis (Thread-worm).—Male, 4 mm. long; female, 10 mm. Head similar in both sexes, with a remarkable cuticular enlargement and three small knob-like lips. Tail of male has six pairs of papillæ. The worms inhabit the large intestine of children, and cause anal itching.

(B) Family Strongylidæ.

Ankylostomum duodenale vel *Dochmius duodenalis* vel *Strongylus duodenalis.*—A most important and widespread parasite occurring in the jejunum, causing the formidable disease Egyptian chlorosis. Found in Italy, Germany, Hungary, Switzerland, Belgium, United Kingdom (Cornish miners) and Egypt. The worm is cylindrical; the male measures 8 to 12 mm. long, the female 10 to 18 mm. Anterior end pointed and bent dorsally. Oral orifice has four claw-like teeth. Tail of male expands into umbrella-like pouch, with three flaps. Tail of female pointed and conical; vulva behind middle third of body. Worms not found in stools unless anthelmintics are given, of which ethereal extract of male-fern or thymol are best. Eosinophilia (in the blood-stream), an important clinical symptom of this disease. For a full description of the disease, see pp. 125 to 129.

(C) Family Trichotrachelidæ.

1. *Trichocephalus dispar* (Whip-worm). — Whip-like hinder end; stout anterior end; filiform. Male 40 mm., female 50 mm. long. Inhabits cæcum.

2. *Trichina spiralis.*—Occurs in different forms in the muscles and in the intestine. From the intestine, where it breeds, it rarely passes out in the fæces unless after anthelmintics. The male is 1·5 mm. long and the female 3 mm. The male has four prominent papillæ between conical elevations at head end. The ova develop into embryos within the uterus and then penetrate within the intestinal walls, to become embedded in the muscles.

(D) Family Rhabdonema (Anguillula) Strongyloides.

1. *Anguillula stercoralis* vel *intestinalis.* — Occur in Cochin China diarrhœa. The immature form has a round body with faint transverse striation. Head a blunt cone, sessile on body, has two lateral jaws, with two teeth in each. The adult measures 2·25 mm. long; the mouth is

triangular, with three lips. It lives in the small intestine. The young worms are passed in the fæces, and infect man without an intermediate host.

Ova of Round Worms and Anguillula.

1. *Ascaris lumbricoides.*—Nearly round, brownish in colour, diameter 0·6 mm.; albuminous outer layer, inside this a tough shell. Contents granular.

2. *Ascaris mystax.*—Globular, larger than *A. lumbricoides*, and finely grooved.

3. *Oxyuris vermicularis.*—Irregularly oval, measure 0·05 by 0·02 mm. Shell membranous, with two or three laminæ. Contents coarsely granular, or often contain a coiled-up embryo.

4. *Ankylostoma duodenale.*—Smooth, oval; measure 0·05 by 0·03 mm. Contain two or three large segmentation spherules. Embryos develop rapidly outside body.

5. *Trichocephalus dispar* (Whip-worm).—Brown; size 0·06 by 0·02 mm. Shell shows a double contour; is flattened at each end with a lid. Contents granular.

6. *Anguillula* (Cochin China Diarrhœa). — Resemble *Ankylostoma duodenale*, but are longer, more elliptical, and pointed at the poles.

Insects' larvæ, taken with food (cheese and meat), may pass out in the fæces. The more important are the immature cheese maggot (*Piophila casei*) and the chrysalis (pupa) form of *Drosophilia melanogastra*, derived from curdled milk.

THE URINE.

Fresh normal urine is free from fungi, but when allowed to stand they rapidly develop. If fermenting urine shows *yeast fungi* in large numbers, glycosuria is suggested. The *Micrococcus ureæ*, in long chains, sarcinæ and numerous other micro-organisms, all occur in decomposing urine.

Pathogenic Organisms.—These may occur in connection with Malta fever, enteric fever, tuberculosis, ery-

sipelas, relapsing fever, ulcerative endocarditis, septicæmia, and plague. As most of these have already been described under their specific headings, a reference to their respective chapters will suffice.

Bacillus typhosus.—Chapter V., pp. 83 to 91.

Micrococcus melitensis.—Chapter V., pp. 91 to 95.

Bacillus tuberculosis.—Chapter IV., pp. 78 to 82.

Spirochæta of Relapsing Fever.—Rare in urine and when present is due to renal hæmorrhage. Chapter II., pp. 27 to 29.

Plague bacillus.—In nephritis. Chapter V., pp. 95 to 99.

Erysipelas.—The urine is generally turbid, and the *Streptococcus pyogenes* or *erysipelatis* may be present.

In examining urine for pathogenic bacteria, it should be collected as sterile as possible, the meatus being disinfected, and the first flow of urine let run waste. It should then be allowed to stand in a large covered conical sterile glass, and the sediment collected and centrifuged. The sediment may be stained by the Ziehl-Neelsen method for *tubercle*, or inoculation of animals undertaken. In the case of the *Bacillus typhosus*, plate culture on Fawcus's or other coli and cocci inhibitory media is the most suitable procedure.

Animal Parasites.

Bilharzia hæmatobia (*Schistosomum hæmatobium*).—The *ova* of this fluke mixed with red blood corpuscles, and perhaps pus cells, sometimes occur in the urine. The ova are easily discernible under a low power, and are pyriform in shape, measuring 120 to 190 μ long by 50 to 73 broad, having a sharp spine at their narrow end. For a full description of the life-history of this parasite and Schistosomum disease, see Chapter II., pp. 35 to 38.

Filaria sanguinis hominis.—This is one of the parasites causing tropical hæmaturia, and filariæ may at times be found in the urine, accompanied by red blood-cells and pus. The characteristics of filaria are described in Chapter II., pp. 38 to 42.

Echinococcus.—The hooklets or fragments of the cyst wall of echinococcus rarely occur in the urine, but are generally derived from rupture of a cyst in the vicinity of the urinary system. Blood-corpuscles are usually associated with their presence. A description of the hooklets and cyst walls will be found on p. 118.

Ascarides.—These very rarely occur and are derived by some abnormal communication between the bladder and intestine. For details concerning Ascarides, see p. 120.

MILK.

Human milk under pathological diseases affecting the mother may contain pus, *staphylococci*, *micrococci* (puerperal septicæmia), *Bacillus tuberculosis*, and in Malta fever the *Micrococcus melitensis*. It is probable that the *Bacillus typhosus* may also occasionally occur. The examination of the milk of wet-nurses should be a matter of importance. The best method of examination is by sedimentation in a large conical glass and centrifuging the sediment, plate cultures from the sediment or inoculation in animals being finally made.

VAGINAL SECRETION.

Numerous yeasts and fission fungi occur, thrush fungi occasionally, *Staphylococcus pyogenes albus*, *citreus*, and *aureus* frequently. Pathologically, the *Bacillus tuberculosis* and the *gonococcus* may be found. An oval infusorial parasite, *Trichomonas vaginalis*, about 10 μ long, with a long tail, three flagella, and lateral cilia, also occurs.

FALLOPIAN SECRETION.

For purposes of diagnosis, the contents of abscesses of the Fallopian tubes removed by operation are important. In them pus may be formed by gonococci, streptococci, staphylococci (rare), tubercle, pneumococci, pneumobacilli, and actinomyces.

BACILLUS COLI COMMUNIS.

This is the most usually isolated intestinal organism. As a causal agent of various inflammatory and suppurative conditions connected with the abdominal and pelvic cavities, it has considerable surgical significance. It is easily isolated from the stools by any of the ordinary methods. Microscopically, although somewhat shorter and less motile than the typhoid bacillus, it is indistinguishable in appearance or by staining reactions. Diagnosis depends on its negative reaction to the Widal agglutination test with typhoid serum and to its characteristic reactions with the following special culture methods:

If inoculated on bile-salt-lactose neutral-red agar plates, and incubated at 37° C. for twenty-four hours, red colonies appear if lactose fermenters (which include B. coli) are present. Inoculation is made from these colonies into the '**flaginac media**,' which are:

(a) *Neutral red-glucose agar*—gas and fluorescence after five to seven days ... ... **Fl.**
(b) *Lactose peptone*—acid and gas ... ... **Ag.**
(c) *Peptone and salt*—indol formed on addition of H_2SO_4 and KNO_2. ... ... **In.**
(d) *Litmus Milk*—acid and clot forms ... **Ac.**

OTHER CHARACTERISTICS OF B. COLI COMMUNIS:

1. Gas formation in gelatine at 20° C.

2. Brown growth on potato at 20° C. Less motile, shorter and stouter; less flagella than *B. typhosus*.

3. Gram-negative.

4. Easily differentiated from *B. typhosus* on Fawcus's medium.

ANKYLOSTOMIASIS.

This disease is characterized by a profound anæmia and eosinophilia, and is caused by a multiple invasion of the intestine by blood sucking nematode worms. The parasite is known as *Ankylostomum duodenale* (vel *Sclerostoma*

duodenale, vel *Dochmius* vel *Strongylus duodenalis*), and is a true round-worm, or nematode, which occurs in nearly all tropical and subtropical countries (Egypt, India, Ceylon, Straits, Java, Japan, West Indies, Guiana, Brazil) and warmer parts of Europe and America. Ankylostomiasis is very common in Egypt, giving rise to the so-called 'Egyptian chlorosis,' and in Italy amongst furnace workers. It caused much trouble in 1880 amongst the workmen in the St. Gothard Tunnel. In 1902 Boycott and Haldane discovered it amongst Cornish miners. The worm occurs in the jejunum, and appears to be parasitic only to man; its ova escape in the fæces.

MICROSCOPIC DIAGNOSIS, ETC.—Anæmia, with true diminution of hæmoglobin and reduction of the colour index. Blood-films will show a high degree of eosinophilia in the blood. Examination of the stools will show the characteristic ova, or after anthelmintics the dead adult worms may be detected in the fæces by diluting them and passing through a fine strainer. The worm, being a nematode, has two sexes.

The Male has a little thread-like body, $\frac{1}{4}$ inch long. The head ends in a cup-shaped disc, bent sharply on itself towards the dorsal surface of the body. Set round the head there is an upper set of four hooks, which it uses for anchoring itself to the mucous membrane, and lower down, at the pharyngeal opening, there are two other hooks, which are used for incising the bloodvessels from which it sucks blood. The tail is characteristic, being expanded in an umbrella-shaped form, containing a bursa with eleven ribs, also the openings for the genitals and excretal aperture, a retractile penis and a long bifid spicule, which acts as a sort of arm during coition.

The Female is a longer and thicker worm, the head being similar to that of the male. The tail, however, differs markedly, as it ends in a point (like an earth-worm), curved towards the dorsal surface. The sexual orifice consists of a vulva with a double vagina, and its orifice is

towards the central portion of the body, at the junction of the middle and posterior thirds and not at the tail. During copulation the worms form a gamma-shaped Y figure.

The Ova, found in the fæces are characteristic, being large oval-shaped bodies 55 μ to 65 μ long by 32 to 40 μ broad, and are surrounded by an oval, clear, transparent shell, the clear translucent border being diagnostic. The yolk is generally segmented in two or four portions.

The ova are passed in the fæces in a very immature state, being incapable of any further development in the intestines, and they require oxygen, moisture and warmth for further growth. They will develop rapidly in warm, damp, moist earth. The yolk enveloping the young embryo bursts from its case and becomes actively motile, swimming about in fluid. If kept under suitable conditions, it increases in size, sheds its skin and develops a gastro-intestinal tract. As far as they have been traced from this stage outside the body is to a form of encystment, and nothing further is known. They can live in water for several hours, but soon die.

CHANNELS OF INFECTION, ETC.—For a long time they were supposed to invade man by being drunk in water; then Looss of Alexandria, while working with a test-tube containing embryos, having spilt it over his hand, felt a tingling, and developed ankylostomiasis. He then spilt some over the leg of a boy who was about to have the limb amputated for some other malady. After operation he examined the healthy skin, and found the embryos had penetrated it through the hair-follicles into the subcutaneous tissue. This mode of infection through the unbroken skin has since been amply confirmed by many experiments, and other varieties of ankylostomata which infect dogs have been proved to be infectious in the same way. After infection they wander about the connective tissues, and cause itching eruptions, called 'ground itch' or 'bunches' by the Cornish miners. Others get into the

lymphatics, many getting destroyed in the lymphatic glands; others pass on to the thoracic duct and get from the blood into the lung vesicles, working up the bronchi to the pharynx and down to the upper part of the jejunum (not the duodenum, as their name would imply). There are three possible routes by which the ova gain access to the blood.

1. By lung to pharynx and intestine, where they shed their skin and mature.

2. Swallowed in water, food, etc.; possible, but not confirmed.

3. Penetration through unbroken skin, the commonest method.

Ankylostomiasis is common in mines, causing a very severe type of anæmia, and is brought about by a large number of parasites (100 or more) hanging on to the mucous membrane of the jejunum. They puncture the small vessels, extracting fluid from the blood, not damaging the red cells, and eat away the epithelial cells of the mucous membrane, frequently changing their site. Punctiform hæmorrhages indicate the position of old bites. The head of the worm contains toxic glands, which, if extracted and injected, cause an anæmia similar to that produced in ankylostomiasis. This anæmia is of peculiar type, resembling chlorosis in that there is a true reduction of the colour index of the blood, this falling as low as 5, and no diminution of corpuscles being coexistent. The reduction of hæmoglobin is marked at times. Poikilocytosis is comparatively rare. Sometimes a degree of leucocytosis may occur, but it is not marked. Eosinophilia is a feature of the disease, it being up from 30 to 60 per cent. A high degree of eosinophilia is said to be a favourable indication of a reaction occurring, tending to get rid of the parasite. Fatty degeneration of the heart may also occur. The mortality of the disease is said to be from 5 to 10 per cent.

PROPHYLAXIS, ETC.—This is of importance, especially in the case of mines, in which often 70 to 80 per cent. of

the mining population may become infected. A systematic examination of the blood for eosinophilia should be made, and, if present, the stools of these individuals should be examined. Miners should be enforced to use latrines, and promiscuous defæcation forbidden; no moist or wet system of disposal of excreta should be adopted, as the ova require moisture for development. No infected man should be allowed to return to work until cured. As regards treatment, thymol is a specific. Put patient in bed and on liquid diet for a few days; give 4 grains of calomel, and the following morning give 30 grains of thymol every hour for three or four doses, avoiding solvents (alcohol, ether, glycerine, turpentine, chloroform, and oils) during treatment.

TRICHINOSIS.

This is a parasitic infection of muscle fibres due to the encysted larvæ of a nematode worm, the *Trichinella spiralis* (syn. *Trichina spiralis*). The normal host of the adult worm is the rat, whose muscles may teem with trichinæ. The geographical distribution of *T. spiralis* is widespread, but human infection is limited to those places where pig's flesh is eaten. Pigs not only kill rats to eat, but also eat stray dead rats, and thus become infected. Rats travel long distances, and carry the disease over wide areas; other animals and rats eat dead rats; pigs eat the excreta of infected pigs, which is in itself infective, and thus the disease is kept going. The male parasite measures 14 to 16 mm long and 0·04 mm. in diameter, its anterior end is tapered and the posterior end has no spicules, but two conical caudal appendages. The female is viviparous, has one ovary; the vulva is at the anterior end of body; it measures 3 to 4 mm. long.

The Parasite.—The adult worm inhabits the duodenum and jejunum of man and certain mammals (rat, pig, wild-boar, dog, cat, fox, mouse, rabbit, hare, sheep, calf, horse, etc.). The males die off after copulation. The pregnant

female penetrates the mucous membrane, and deposits its young—estimated at least 1,500—in the lymph or blood stream of the intestinal wall, or free in the abdominal cavity, whence they become distributed throughout the entire body; but conditions necessary for further development only exist in the striped muscles, which they invade, causing severe inflammatory reaction, giving rise to pyrexia (102° to 104° F.) and pain by muscular contraction (myositis), so frequently mistaken for rheumatism; finally, true muscle degeneration and œdema occurs, especially under the eyes. The seats of muscular selection are chiefly the psoas muscle, diaphragm, tongue, abdominal, laryngeal and intercostal muscles, and the points of insertion of tendons. In the muscles, after a short rest, the embryo worms coil up and encyst, forming lemon-shaped bodies, the long axes of which lie parallel between the muscle fibres, and measure 4 mm. long by 0·25 mm. broad. After six to nine months calcification, beginning at the poles, spreads slowly, and in lapse of years the trichinæ become calcified themselves. During encystment the worms remain alive, and are capable of further development. In the pig this is estimated at eleven years, and in man twenty-five to thirty-one years. In the pig, as a rule, the capsules are not calcified, and hence are not visible to the naked eye. When meat containing living encysted worms is eaten, the capsules are dissolved and the embryos are set free; passing into the small intestine, they become sexually mature in three days, and parturition takes place in six or seven days; there is, therefore, but a short time for purgatives or vermicides to be of use. The embryos, if born in the intestine, bore their way through the mucous membrane, and enter the venous or lymph channels, passing thence to the muscles; but they are more usually born outside the intestine, by migration of the female worm, as already described.

Blood Examination.—Eosinophilia is a great aid to diagnosis. The outside normal limit of eosinophiles lies

between 0·67 and 11 per cent. (Zappert). They are increased in diseases due to internal parasites, such as trichinosis, ankylostomiasis, bilharziosis, and in certain skin diseases, asthma and emphysema. In trichinosis the eosinophile percentage of all leucocytes may rise to 40 per cent., and even as high as 80 per cent.

MICROSCOPICAL EXAMINATION.—The stools may be examined for adult trichinæ, but as a rule the result is negative. If the remains of the fresh or salted pork eaten by the patient is available, and the muscle fibres be teased or crushed, and examined under a low power, the capsuled embryos show up distinctly. Permanent specimens, which should be fairly thick, may be made by embedding in paraffin and staining with hæmatin solution and eosin, the worm taking up the former and the muscle the latter dye. Frozen sections are useless, as the cysts fall out.

PROPHYLAXIS.—In infected localities, since pickling or smoking pig's flesh does not in all cases kill trichinæ, and since cooking large pieces of this meat does not allow the penetration of a temperature of sufficient thermal death-point to the central parts of a joint, it is necessary to have the animal's muscle (sections of tongue and psoas) microscopically examined by experts after slaughter, as is enforced by law in Germany, trichinous pigs being condemned as unfit for human consumption. Personal prophylaxis will consist in either not eating pork or only eating it when it is very thoroughly cooked.

CHAPTER VIII

STAINING PROCESSES AND REAGENTS — AGGLUTINATION REACTIONS—THE EXAMINATION OF PATHOLOGICAL FLUIDS — OPSONIC INDEX — BLOOD-SUCKING INSECTS—VACCINE-THERAPY

Stains for Blood-Films.

I. LEISHMAN'S METHOD (a modification of Romanowsky's).

(*a*) If the blood-films are old, restore alkalinity with fresh serum prior to fixing.

(*b*) Place a few brops of the stain on the film to fix it, and let it stand for fifteen to thirty seconds.

(*c*) Add about doudle the quantity of distilled water, gently mix, and let stand for five to fifeetn minutes.

(*d*) Gently wash off stain with distilled water. Alternative washing and drying with filter-paper intensifies the colour contrasts in the various cells. The degree of intensification should be controlled by observations with a low-power objective.

(*e*) Dry between filter-paper and mount. Cedar oil is the best material for mounting in, as it does not cause the films to fade, as Canada balsam, if acid, may do.

The stain is a combined fixing and staining solution, so no separate process of fixation is necessary.

II. EOSIN AND METHYLENE BLUE METHOD.

(*a*) Fix in a saturated solution of perchloride for thirty seconds, or, better still, with methyl alcohol or formalin vapour.

(*b*) Well wash in tap-water.

(*c*) Stain in 1 per cent. watery eosin thirty seconds.

(*d*) Wash in distilled water and dry by blotting.

(*e*) Pass through the flame three to five times to fix the eosin.

(*f*) Counterstain in watery methylene blue half to one minute; use it weak, and examine depth of staining under low-power objective.

(*g*) Wash, dry, mount, and examne.

III. Acid Hæmatein and Eosin Method.

(*a*) Fix in saturated corrosive sublimate (filtered) and wash well in tap-water

(*b*) Stain in hæmatein three to five minutes, keeping the solution warm.

(*c*) Wash in tap-water one m'nute.

(*d*) Counterstain in dilute watery eosin (one drop of a 2 per cent. solution in half a watchglassful of distilled water).

(*e*) Wash, dry, mount, and examine.

IV. By Carbol-Thionin Method.

(*a*) Fix in saturated perchloride, wash thoroughly, and stain for eight minutes in carbol-thionin.

(*b*) Wash well in tap-water to differentiate.

(*c*) Dry, mount, and examine.

Examination of Blood-Smears for Pneumococcus.

Fix in perchloride, wash, stain by Gram's method, counterstain with watery eosin, and examine.

Muir's Method to demonstrate Capsules of Pneumococcus.

Fix in mordant, stain in filtered warm carbol-fuchsin (strong) for thirty to sixty seconds. Rinse in 70 per cent. alcohol. Wash in water; place in mordant solution for sixty seconds; wash well in water. Treat with 70 per cent. spirit for one minute. Wash in water. Counterstain with methylene blue forty seconds; wash, dry, and mount.

Trypanosomata, Malaria, and Kala azar Parasites.

These are best examined fresh, as moist, unstained preparations. Blood-smears and smears of brain tissue or spleen pulp may be stained by Leishman's method. If old blood-films, restore alkalinity with blood-serum before fixing.

Sections of tissues (brain in sleeping sickness or malarial coma) may be stained by Leishman's method for sections, or by the carbol-thionin method.

Filariæ.—As blood-films should be made pretty thick, soak the unfixed films first in distilled water to dissolve out the hæmoglobin; then dry and stain by Leishman's stain.

Bilharzia Ova.—Examine bloody sediment of urine fresh. In sections of the bladder wall, stain by the hæmatein-eosin method.

Leishman's Method of Staining Sections.

(*a*) Mix equal parts of Leishman's stain in distilled water in a watch-glass.

(*b*) Pour mixture on to the section, and stain for fifteen minutes.

(*c*) Decolorize in acetic acid (1 drop of 1 in 300 in a watch-glass of water) until the section turns pink. Wash in tap-water to stop action of the acid.

(*d*) Dry off almost all the water with blotting-paper.

(*e*) Dehydrate very rapidly in absolute alcohol.

(*f*) Clear in xylol and mount in Canada balsam.

Staining Methods for Spirochætæ.

Perfectly clean slides and cover-glasses are necessary, and are best cleansed by absolute alcohol, which is burnt off. Giemsa stain is best for fresh films, and Levaditi's silver method, or its modifications, for sections.

A. Films (Giemsa).*

1. Spread film, dry in air, fix in methyl alcohol for five minutes.

2. Apply 1 drop of Giemsa stain in 1 c.c. water for fifteen to twenty minutes.

3. Wash in distilled water, dry, and mount in cedar oil. If Canada balsam is used, it should be neutral, as acid causes the stain to fade.

B. Sections.

1. Small, thin slices of the affected tissue, 1 mm. thick, are fixed in 10 per cent. formalin for twenty-four hours, then washed in water for one hour, and placed in 96 per cent. alcohol for twenty-four hours.

2. Transfer to incubator at 37° C. for three days in a dark bottle containing 1·5 per cent. solution of silver nitrate.

3. Wash in water for half an hour, and transfer to a dark bottle for two days containing 4 per cent. watery solution of pyrogallic acid to which some 5 per cent. formalin has been added. Wash in water.

4. Transfer to alcohol of three increasing strengths up to absolute alcohol, keeping one day in each.

5. The tissue is then cut in sections and mounted; the spirochætæ appear as minute black spirals against a pale yellow background; the latter may be counterstained with dilute carbol-fuchsin if desired.

Yamamoto has recently introduced a modification of the silver nitrate method, which avoids the disadvantage of staining the tissues as well as the spirochætæ. The tissue to be investigated may be hardened in various solutions, and is then cut into small pieces 10 mm. long and 5 mm. in thickness and breadth. These are washed free from the fixing media by rinsing in water for twenty-four hours and finally in distilled water

* Giemsa stain will also give good results when used for staining blood-films to demonstrate other pathological conditions.

for one hour. Each piece is then put into 10 c.c. of a 5 per cent. solution of silver nitrate in brown-coloured bottles, and kept at 37° C. for forty-eight hours. At the end of this time they are placed in a reducing solution containing 2 per cent. of pyrogallic acid and 1 per cent. of tannic acid in distilled water in similar brown bottles, in which they are kept for twenty-four hours at 37° C. It is, however, necessary to change this solution after the first half-hour, since it is rendered turbid by the reduction. After this the pieces of tissue are placed in water for an hour, and then washed in alcohol of increasing strength until decolorized; they are then embedded in paraffin or celloidin, the latter giving better results. If it is desired to counterstain the tissues, the sections should be dipped in Löffler's methylene blue solution for a second. The celloidin is removed in the usual way, and the specimen cleared by oil of origanum instead of by xylol, and then mounted in balsam.

Staining Spirochæta Pallida.

(A) A Section of Hard Chancre (stained by Levaditi's method).

I. Fix the specimen on a slide with a solution of egg albumen and glycerine.
II. Remove paraffin by xylol.
III. Mount in Canada balsam.

(B) To stain Smear from Hard Chancre.

I. Fix with methyl alcohol half to one minute.
II. Wash in distilled water.
III. Dry.
IV. Apply serum for two minutes.
V. Drain off excess of serum, and, without washing, allow serum to dry thoroughly.
VI. Pour on a mixture of 2 parts Leishman's stain and 3 parts distilled water, taking care not to disturb the layer of serum. Stain for an hour or more, changing the staining mixture occasionally.

VII. Wash and examine.

N.B.—The specimen should be stained until the nuclei of the leucocytes are almost black.

Examination of Pus Film containing Streptococci.

I. Fix in perchloride thirty seconds ; wash well. Stain by Gram's method as follows :

(*a*) Aniline gentian violet, five minutes.

(*b*) Gram's iodine, two minutes.

(*c*) Absolute alcohol, until no more blue colour comes away.

(*d*) Wash well in water and counterstain in carbol-fuchsin one to thirty seconds ; wash in water, dry, and examine.

II. Pus films may also be stained with Leishman's stain, the same as for blood-films (see p. 132).

Tubercle Bacilli.

1. *In Sputum.*—Make films ; fix by heat, and stain by Ziehl-Neelsen's method as follows :

Carbol-fuchsin hot ten minutes.

Decolorize in 25 per cent. sulphuric acid.

Wash well.

Counterstain in watery methylene blue for thirty seconds.

Examine.

2. *In Pus.*—Make films ; fix in corrosive sublimate ; wash ; stain by Ziehl-Neelsen's method ; counterstain ; examine.

3. *In Sections.*—Stain paraffin sections by Ziehl-Neelsen's method, using counterstain for two minutes.

Cytological and Bacteriological Examination of Sputum.

In order to carry out, by means of one film, a cytological as well as a bacteriological examination of sputum, Eckenstein has described a combined method of staining, which he claims to be of considerable value. Films are care-

fully made from recent sputum, precautions being taken to avoid crushing. They are stained with carbol-fuchsin, and gently heated in the usual manner. Decolorization is effected by means of 20 per cent. sulphuric acid, and the specimen is washed in 95 per cent. alcohol and in distilled water. It is counterstained for one or two minutes in a solution of the precipitated stain of Giemsa dissolved in methyl alcohol, then a few drops of distilled water are added, and the specimen is shaken for about a minute, and then washed in distilled water. The stains of Jenner, May, Grunwald and Leishman give similar results, and they may be combined with ordinary Giemsa stain diluted to 1 in 20. By this means the various cells —such as lymphocytes, polymorphonuclear leucocytes, and epithelial cells—found in sputum can be differentiated and their percentage determined. Mucus is stained violet, fibrin greenish, and albuminous exudates bluish, while tubercle bacilli are stained red, and other organisms blue. The same process may be applied to other pathological exudates and fluids.

Staining Frozen Sections of Tubercular Leprosy.

Float section on slide, remove water, place piece of cigarette-paper on section, and then, pressing with a layer or so of blotting-paper over this, allow section to dry, fix by heat, and stain either—

I. By Ziehl-Neelsen, decolorizing with 10 per cent. H_2SO_4; or

II. By Gram, counterstaining with dilute eosin.

Examination of Diphtheria Films.

I. By Gram's method (see p. 137).

II. Carbol-thionin, five minutes (p. 133).

III. Neisser's method.

NEISSER'S METHOD.

Two parts of 'A' solution and one part of 'B' solution for three seconds; wash at once in water.

Counterstain in cresoidin, two seconds; wash, dry, mount and examine.

'A' SOLUTION.		'B' SOLUTION.	
Meth. blue powder	1 grm.	Crystal violet ...	1 grm.
Alcohol absolute	50 c.c.	Alcohol absolute	10 c.c.
Glacial acetic acid	50 c.c.	Aqua destill. ...	300 c.c.
Aqua destill. ...	1,000 c.c.		

COUNTERSTAIN.—Cresoidin 1 part dissolved in warm water 300 parts, and filtered.

Plague.

Examination of splenic smears for *B. pestis.*

1. Stain by carbol-thionin for ten minutes (p. 133).
2. Examination of broth culture of *B. pestis.*
   (*a*) Make films, fix, clear. Stain by carbol-thionin for eight minutes (p. 133).
3. Stain paraffin sections of plague spleen.
   (*a*) By carbol-thionin (p. 133).
   (*b*) By Leishman (p. 132).

Cholera.

1. Make hanging-drop.
2. Make film, and stain by carbol-fuchsin 1 in 10 for five minutes.
3. Make stab preparation on gelatine.

Cholera liquefies, but not so rapidly as Finkler-Prior. Easy to diagnose. Take a flake from rice-water stools on slide and stain ; look for 'fish-in-stream' appearance. The cholera vibrio grows quickly in 1 per cent. peptone, 5 per cent. salt ; is an aerobe, and grows on surface. Cholera vibrio dies out very quickly in well-water after infection, so do not dilute it, but take some of the water and add salt and peptone to it, and incubate. Remember that Finkler-Prior's organisms are pathogenic for fowls, which may infect well and other water.

Agglutination Test for Typhoid Fever (Widal's Reaction) with Serum from a Rabbit immunized against Typhoid.

Widal's reaction will not occur in blood that has been boiled or roasted. This should be remembered when

sealing glass tubes containing blood to be examined. The Widal reaction is carried out as follows:

A. Make a series of dilutions of the serum in normal salt solution.

1	vol	serum + 4	vol.	NaCl	= 1 in 5 dilution.
1	,,	1-5 + 1	,,	,,	= 1 in 10 ,,
1	,,	1-5 + 4	,,	,,	= 1 in 25 ,,
1	,,	1-25 + 1	,,	,,	= 1 in 50 ,,

Mix each dilution, and blow out into separate watch-glasses; label, and cover each with another watch-glass.

B. From dilutions of serum fill four sedimentation tubes by mixing in each case 1 volume of diluted serum with 1 volume of emulsion of typhoid bacilli.

Allow an unbroken volume of the mixed serum and emulsion, about 6 inches long, to run up into a clean pipette, and seal off the tube in a flame.

Note that this addition of an equal volume of emulsion to the diluted serum doubles the total dilution of the serum, and label your tubes accordingly—1 in 10, 1 in 20, 1 in 50, 1 in 100.

Put up a control tube of 1 volume of emulsion and 1 volume of NaCl. N.B.—The control tube *must* contain no serum, and should be put up before the serum tube is broken.

The standard laid down for Widal's reaction *under the microscope* is complete agglutination in half an hour with a 1 in 20 dilution and for *sedimentation tubes* twenty-four hours. After twenty-four hours examine the sedimentation tubes put up, contrasting the serum tubes with control.

Using the same serum as before, prepare the sedimentation tubes with higher dilutions of serum to determine the limits of agglutination.

1. Prepare dilutions of 1 in 100, thus—

1	vol.	serum + 9	vol. of	NaCl	= 1 in 10 dilution.
1	,,	1-10 + 9	,,	,,	= 1 in 100 ,,

2. From the 1 in 100 dilution prepare the following—1 in 200, 1 in 300, 1 in 500.

3. From these four dilutions—1 in 100, 1 in 200, 1 in 300, 1 in 500—put up sedimentation tubes of 1 in 200, 1 in 400, 1 in 600, and 1 in 1,000.

4. Put up control.

Agglutination Test with Micrococcus melitensis.

1. Put up control.

2. Make the following dilutions of serum—1 in 10, 1 in 25, 1 in 50, 1 in 200, 1 in 400, and 1 in 500.

3. From the above dilutions put up sedimentation tubes of 1 in 20, 1 in 50, 1 in 100, 1 in 400, 1 in 800, 1 in 1,000. In some patients 1 in 3,000 will agglutinate.

Take 1 volume of emulsion (always take up emulsion first), add 1 volume of serum diluted 1 in 25, mix, and examine a hanging-drop preparation, and compare with control.

4. To stain the bacilli, make a film of the emulsion, fix and stain with carbol-fuchsin 1 in 10 for five minutes.

Staining Paraffin Section of Typhoid Spleen.

This may be carried out either:

I. By Leishman's stain; or,

II. Carbol-thionin, eight minutes.

After staining in thionin, differentiate and dehydrate in alcohol, clear in xylol, and mount in Canada balsam; the section should be a pale green colour.

Ziehl-Neelsen's Method of Staining Acid-Fast Bacilli.

Carbol-fuchsin	Basic fuchsin	... ...	1 part.
	Abs. alcohol	... ...	10 parts.
	Sol. carbolic acid (1 in 20)		100 ,,

Fix by heat.

1. Place specimen in this fluid, heat till steam arises, and allow to remain in it for ten minutes.

2. Decolorize with 25 per cent. watery solution of strong HCl, H_2SO_4, or HNO_3, until the tissues become yellow.

3. Wash well with water. Tissues regain a faint pink

tint. If colour is distinctly red the decolorization is not sufficient, and the specimen must be returned to the acid, and removed from the acid every few seconds and washed in water until the proper pale pink tinge is obtained.

4. Counterstain with solution of methylene blue for half a minute.

5. Wash well in water. If films, dry and mount; if sections, dehydrate with absolute alcohol, clear with xylol, and mount.

Staining Paraffin Sections by Gram's Method.

During the entire process the section should not be allowed to dry.

A. Prepare the section for staining.

I. Warm the section in a watchglassful of water; to smooth out the wrinkles.

II. Transfer the section by tissue-paper to a slide prepared with fixing fluid (equal parts of egg-albumen and glycerine).

III. Fix the section by coagulating the albumen by gentle heat.

IV. Dissolve out the paraffin by xylol, and get rid of latter by washing in absolute alcohol.

V. Wash in water to remove alcohol.

B. Staining the section.

I. Stain by Gram's, and counterstain in watery eosin or carbol-fuchsin 1 in 10 (thirty seconds).

II. Stain by Leishman's method of staining sections. Mix 1 volume of stain with 1 volume of distilled water in a watchglass.

Pour mixture on section, and allow to stain for fifteen minutes, until deep blue.

Decolorize in weak acetic acid (1 drop 1 in 300 in half watchglassful of water) until *almost all* the blue has come out and the section is of a mottled-pink colour. Wash in water to stop the action of the acid. Dry off almost all the water by blotting.

C. Prepare the section for mounting.

Dehydrate (rapidly in the case of Leishman's stain) in absolute alcohol, rapidly follow by clearing in xylol; mount in Canada balsam.

Stain for Spores (Anthrax).

1. Make films, fix by heat, pass twelve or fourteen times through flame, clear.

2. As stain evaporates add 1 in 20 acid carbolic, *not* more stain. Stain in hot carbol-fuchsin twenty minutes.

3. Decolorize in 1 per cent. sulphuric acid until rods are faint red and spores bright red. Wash to stop acid.

4. Counterstain in watery methylene blue thirty seconds. Wash, dry, mount and examine. Spores are stained red, bacilli blue.

The Examination of an Unknown Culture or a Pathological Fluid.

1. State the naked-eye appearance—whether clear, turbid or flocculent—and its colour.

2. Make a hanging-drop. State if bacilli or cocci are present, and whether motile, in pure or mixed culture or not. Presence or absence of refractive spores.

3. Make a film, fix by heat, stain by Gram's method, and counterstain. State the results.

4. See if acid-fast stain by Ziehl-Neelsen method; and, if necessary, stain by Neisser's method and also stain for spores.

5. Dilute the culture in a tube of sodium chloride in distilled water, and from this make an agar plate cultivation. Examine this in twenty-four hours, and describe the colonies, which should then be 'fished,' and hanging-drop and stained preparations made from a colony of each kind. Then make smears, and stain them by Gram's method with counterstain.

6. From a colony containing motile Gram rods, if present, make a culture into a tube of broth, and examine this in twenty-four hours, as follows:

(*a*) Stain a specimen by Gram with counterstain.

(*b*) Test the broth culture with antityphoid and other seia.

Prepare $\frac{1}{25}$ serum with broth culture.

Compare with a control containing equal volumes of saline and culture.

To estimate the Opsonic Index of Serum for Staphylococcus Albus.

A. First prepare a quantity of washed blood cells as follows: Receive a quantity of washed blood from the finger in 1 per cent. citrate of soda in saline, mix, centrifuge till supernatant fluid is clear, withdraw supernatant fluid and replace with saline, mix, centrifuge as before, replace supernatant fluid with saline, and again centrifuge.

B. Prepare an emulsion of the organism in saline, centrifuge to remove clumps, take up the upper homogeneous portion of the emulsion into a fresh tube.

C. Patient's serum and the serum of a normal person should be taken at the same time, and should not be more than forty-eight hours old.

D. Make the following mixture:

Washed blood-cells, 1 volume
Emulsion of bacteria, 1 volume

Mix well, take up an unbroken column of the mixture into a pipette, seal off the end, incubate for fifteen minutes at 37° C., spread a film with an edge, stain by Leishman for five minutes. Count the number of bacteria taken up by fifty polynuclear leucocytes, and calculate the average per cell.

The opsonic index: $\frac{\text{Patient}}{\text{Norma}}$, *e.g.* $\frac{8}{10}$; opsonic index 0·8.

Blood-Sucking Insects.

The more important of these are the mosquitoes, *Culex fatigans*, spreading filariasis, the *female anopheles*, carry

ing malarial fevers, *Stegomyia fasciata*, associated with yellow fever; and *Glossina palpalis*, the propagating agent of sleeping sickness.

The *female anopheles* is distinguished from the male in having a few short hairs on the antennæ, whereas the male has long feathery curled antennæ (whiskers). The palpi which lie inside the antennæ should not be mistaken for these.

DIFFERENCES BETWEEN *Culex* AND *Anopheles* MOSQUITOES TABULATED.

Culex.	Anopheles.
Female. — A hump-backed gnat, with its head set at an angle.	*Female.*—A straight gnat; head in line with body; stands like a peg on the wall.
Palpi quite short knobs.	*Palpi* as long as proboscis.
Eggs. — Laid in masses, floating like rafts on stagnant water.	*Eggs.* — Boat-shaped and laid crosswise in pairs; found in fresh or running water.
Larvæ. — Swim near top, but lie obliquely to surface of water. Has long respiratory tube.	*Larvæ.* — Lie parallel to surface of water; breathe through aperture in tail; have eight abdominal segments and hairs, which agitate surface of water.
Pupæ.—Hump-backed.	*Pupæ.*—Head smaller.

Glossina palpalis.—Distinguished by having its wings folded quite close (like scissors) to the body when at rest, and by the characteristic venation of the wings. It has a proboscis with an onion-shaped growth at the base, which represents the two palpi fused into a sheath.

VACCINE-THERAPY.

Before considering the subject of vaccine-therapy, a word or two on immunity in general is necessary to show the relationship that the former bears to the latter.

VARIETIES OF IMMUNITY.

According to the means by which it is produced, artificial immunity may be said to be of two kinds :

1. ACTIVE IMMUNITY.—This is the immunity an individual man or individual animal produces by himself for himself.

2. PASSIVE IMMUNITY.—This is the immunity produced in a man or in an animal by injecting the blood-serum of an animal that has been rendered immune by active immunity described in (1).

Passive immunity does not concern vaccine-therapy, and may be left out of further consideration ; we can, therefore, pass on to consider active immunity.

Active Immunity causes two classes of substances to form in the blood :

(*a*) Antitoxic.

(*b*) Antibacterial.

The antitoxic substances neutralize the toxins formed by bacteria, and as they do not concern vaccine-therapy they may be dismissed from further consideration.

The antibacterial substances bring certain agencies into play :

(*a*) Agencies connected with leucocytosis and concerning phagocytosis.

(*b*) The production of antibacterial substances in the blood. Antibacterial substances are produced in the blood in the presence of all kinds of bacterial injections. How many different kinds of antibacterial substances there actually may be is not known, but there are at least four. There are :

1. Agglutinins.

2. Bacteriocidins, which kill bacteria.

3. Bacteriolysins which not only kill the bacteria, but break them up.

4. Opsonins, which act directly on the bacteria, and make them very much more susceptible to phagocytosis than is normally the case.

Agglutinins, bacteriocidins, bacteriolysins, and opsonins, appear to be quite distinct substances, and serum can be obtained with one or more of these substances absent; for instance, agglutinins may be present and there may be no opsonins, etc. All, however, combine to effect the destruction of bacteria and bring disease to an end. If they are ineffective, the result is a local death of the tissue affected or a general death of the body.

Of the four substances, the opsonins are the most important. All bacteria tested are susceptible to this substance. The action of the others is not so constant, and is only demonstrable in certain diseases.

OPSONINS.—As defined by Sir Almroth Wright, these are substances found in the blood which act directly on bacteria, making them suitable for phagocytosis. If the bacteria are not acted on by the opsonins, they may be phagocyted, but this is not so likely to occur to the same extent as if not acted upon by the opsonins.

Opsonins are present to a small extent in normal blood, but when disease progresses they increase. Like all antibodies, opsonins respond to a stimulus, and are called out by the presence of bacteria. By using vaccines we can call out the opsonins.

VACCINES.—The best form of vaccines is probably not yet known, but if germs are killed by heat and their dead bodies injected, an efficient vaccine is made. All vaccines must, however, be standardized before injection, and this is done by counting the actual number of germs and regulating the dose to be injected accordingly.

METHOD OF ASCERTAINING THE IMMUNIZING VALUE OF A VACCINE.

This is very complicated, from the fact that a host of antibodies are produced by the injection of any vaccine into the body, and it is not known which of these is the important one to select for the purpose of the test. Opso-

nins, however, furnish the best test of efficiency of a vaccine.

To Estimate the Opsonic Index.

Two estimations must be made—first, the phagocytic power of your own leucocytes in your own serum, and secondly the phagocytic power of your own acting in the patient's serum, under the same conditions and using the same germs. Colonel Sir William Leishman, however, strongly insists on using the patient's leucocytes with his own (the patient's) serum against the observer's leucocytes and the observer's serum.

Example.—Suppose that on an average three cocci are found in each leucocyte acted on by the patient's serum, and six in each leucocyte acted on by the observer's serum, the opsonic index is said to be three over six, or one-half.

Opsonic Changes occurring in the Blood as a Result of Inoculation by a Vaccine.

Very different reactions occur, dependent on the amount of vaccine injected, which may be an under-dose, an over-dose, and an appropriate dose.

An Over-dose lowers the opsonic index and produces a negative phase; the negative phase may recover itself and the opsonic curve rise, but it will not rise above normal.

An Appropriate Dose.—This produces a slight negative phase, followed by a rise (positive phase), which falls a little and then tends to keep high for some time. Wright calls this the high-tide curve.

An Under-dose.—This causes a very slight rise, which is not maintained and which soon falls, causing no clinical improvement in the patient, showing that small doses are of no benefit.

If repeated injections are necessary, Wright recommends that the opsonic index should be estimated every

few days, and the injections made only during a positive phase. After giving an injection, estimate the opsonic index twenty-four hours after, and again after a week.

If the opsonic index is found twenty-four hours after injection to be considerably reduced, it is evident that an over-dose has been given, and it is wise to reduce it. If, however, there is a small rise, and if after waiting a week it is not standing higher than before the last injection, it indicates that the dose is too small, and points to the necessity of increasing it. If, on the other hand, a fall is found after the first twenty-four hours, and a good rise is perceived a week later, it indicates that a proper or appropriate dose has been given.

Serum-therapy is a difficult and laborious process, requiring the utmost skill and practice in its technique ; for this reason many have failed to obtain good results, and consequently have attempted to throw discredit on its advantages. Auto-inoculation by bacteria in the body and the presence of different organisms acting in symbiosis have also tended to complicate matters for observers working at serum-therapy, so that a few remarks on these subjects will not be out of place.

AUTO-INOCULATION.—This occurs when bacteria get moved from the portion of the tissue in which they are acting (or perhaps dying off) to another portion o fresh tissue. It may occur accidentally or otherwise, and the germs, getting upon a fresh culture medium, so to speak, renew their vigour, and produce a renewal of the disease process. Auto-inoculation frequently occurs during different diseases—*e.g.*, a case of tubercle of the lung—and it can be produced experimentally in tuberculosis and gonorrhœa of joints by massage. Auto-inoculation may call forth opsonins, and massage and heat, by producing an increased flow of blood to the part, may help towards a cure. If this be so, why not employ auto inoculation in the treatment of disease? The reasons against its employment are as follows :

(*a*) Nature frequently fails in employing it, because no regular dose can be estimated, and too small or too large a dose of organisms may be dislodged.

(*b*) Here we are dealing not only with dead organisms, but with living bacteria, which, by getting into the circulation, may produce a septicæmia.

We may ask ourselves how it is that the bacteria in disease should of themselves not call forth the production of opsonins, and why should not opsonins kill the bacteria?

At the onset of a disease the opsonins may be high, and owing to the bacteria being protected by pus, necrotic tissue and ascitic fluids, etc., which have neither any opsonic power nor the power of allowing penetration to the opsonins, they fail to reach the bacteria. The lymph stream must reach the organisms, and in necrotic tissue tryptic ferments form, and act as a serious barrier to the flow of lymph. Hence the surgeon steps in and removes the pus, and encourages the flow of lymph by heat and fomentations, and the opsonins finish the healing process by producing a fertile soil for phagocytosis. In some forms of 'brawny swelling,' associated with a high clotting power of the blood, Sir Almroth Wright recommends the use of a warm bath of citrate of soda $\frac{1}{2}$ per cent. and normal salt 5 per cent., to increase the blood-flow, and this has had wonderful results.

Class of Cases suitable for Vaccine-Therapy.

Localized bacterial affections, involving the subcutaneous tissues, such as abscesses, boils, tubercular glands, inflammatory diseases of bone and joints, and ulcerative injections of all sorts.

Mixed infections present difficult problems. A mixed vaccine may, however, be employed to counteract them. Tuberculosis is particularly troublesome for this reason. A tubercular cavity in a lung has many forms of organisms acting on its walls, and may be regarded as an open ulcer.

Systemic or septicaemic infections have also been treated by vaccine-therapy, and some of the most desperate conditions, such as malignant ulcerative endocarditis, have done well. In all streptococcic infections a daily estimation of the opsonic index is absolutely necessary, as the vaccines may otherwise produce dangerous and disastrous effects.

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THE END

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